

ADRENAL CORTEX

Transactions of the Fourth Conference
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ERRATA

Transactions of the Third Conference on Adrenal Cortex

- 1 Table X on page 112 for orally read intramuscularly
- 2 In Figure 33 (page 138) and Table XVIII (page 142) and throughout the presentation of Oscar M. Hechter The Biogenesis of Adrenal Cortical Steroids (pages 115 through 155) for pregnenolone read pregnenolone.

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JOSIAH MACY, JR FOUNDATION CONFERENCE PROGRAM

AS AN INTRODUCTION to these Transactions of the Fourth Conference on Adrenal Cortex I should like to outline what it is that the Foundation hopes to accomplish by its Conference Program. We are interested first of all in furthering knowledge about the adrenal cortex and to this end the participants were brought together to exchange ideas, experiences, data, and methods. In addition to this particular goal, however, there is a further and perhaps more fundamental aim which is shared by all our conference groups. This is the promotion of meaningful communication between scientific disciplines.

The problem of communication between disciplines we feel to be a very real and urgent one, the most effective advancement of the whole of science being to a large extent dependent upon it. Because of the accelerating rate at which new knowledge is accumulating and because discoveries in one field so often result from information gained in quite another, channels must be established for the most effective dissemination and exchange of this knowledge.

The increasing realization that nature itself recognizes no boundaries makes it evident that the continued isolation of the several branches of science is a serious obstacle to scientific progress. Particularly is it true in medicine that the limited view through the lens of one discipline is no longer enough. For example, today medicine must be well versed in nuclear physics because of the tracer techniques and the injury which can result from radiation. At the other extreme, medicine is certainly a social science and through mental health must be concerned with economic and social questions. The answer then is not further fragmentation into increasingly isolated specialties, disciplines, and departments, but the integration of science and scientific knowledge for the enrichment of all branches. This integration we feel can be encouraged by providing opportunities for a multiprofessional approach to given topics.

Although the fertility of the multidiscipline approach is recognized, adequate provision is not made for it by our universities, scientific societies, and journals. And perhaps the presence of other hindering factors must be admitted. Partly semantic in nature, they may also to

some degree be psychological. Admittedly it is oftentimes difficult to accept data derived from methods with which one is unfamiliar. By making free and informal discussion the central core of our meetings we hope to achieve an atmosphere which minimizes as much as possible these semantic and emotional barriers.

Thus our conferences are in contrast to the usual scientific gatherings. Presentations are not designed to present neat solutions to tidy problems but rather to elicit provocative discussion of the difficulties which are being encountered in research and practice. We ask that the presentations be relatively brief and emphasis is placed upon discussion as the heart of the meeting. Our hope is that the participants will come prepared not to defend a single point of view but with open minds to take full advantage of the meeting as an opportunity to speak with representatives of other disciplines in much the same way as they talk with their colleagues in their own laboratories.

During 1953 under the Conference Program conferences will be held on the following topics: administrative medicine, adrenal cortex, aging, connective tissues, consciousness, cybernetics, infancy and childhood, liver injury, metabolic interrelations, nerve impulse, renal function and shock and circulatory homeostasis.

When a new conference is organized the Chairman in consultation with the Foundation selects fifteen scientists to be the nucleus of the group which will hold annual meetings for a period of five years. Every effort is made to include representatives from all pertinent disciplines. From time to time however new members are added by the group to fill gaps in viewpoint or techniques. A small number of guests is invited to attend each meeting but for the purposes of promoting full participation by all members and guests attendance at any meeting is limited to twenty five. During a conference's prescribed lifetime we cannot possibly include more than a small fraction of the key investigators in the field and one of the difficulties in forming a group such as this one on the adrenal cortex is that it is necessary to exclude so many investigators we should like to include.

The transactions of these meetings are recorded and published. This is done because the Foundation wishes to make current thinking in a field available to all those working in it and to those in other fields who are concerned with science for example government officials, administrators, etc. Logic is a vital aspect of science but equally essential is the intuitive or creative aspect. Research is as creative as the painting of a portrait or the composing of a symphony. Although logic is of course necessary in order to rearrange, to test and to validate, research thrives on creativity which has its source in unconscious, nonrational processes. Unfortunately however in the research reports which are

presented to the world in scientific journals this integral part of scientific endeavor is shriveled by the cold white light of logic. By preserving the informality of our conferences in the published transactions we hope to portray more accurately what goes on in the minds of scientists and to give a truer picture of the role which creativity plays in scientific research.

FRANK FREMONT SMITH M.D.

Medical Director

PERMISSIVE ACTION OF ADRENAL CORTICAL HORMONES

INFORMAL DISCUSSION

Long / Departing from our usual procedure the discussion at this opening session will not be based on a presentation made by one of the participants but will have as its impetus unfinished business left over from previous meetings of the group. I have re read the reports of the former conferences and have jotted down in no particular order topics that may be interesting to explore further. For example in the discussion of Dr Pitts' paper last year Dr Ingle spoke of what has been termed the 'permissive action' of the adrenal cortical hormones. As I understand it this term means that in the total absence of any adrenal hormones in the body there are a great many actions normally carried out by the cells metabolic processes of one kind or another that either proceed at very slow rate or else exhibit marked deficiencies. The administration of minimal amounts of adrenal hormones—perhaps we can call them basal amounts—enable the cells to carry out a large number of reactions of various kinds which they cannot do in the absence of the hormone.

The point that interests me is the comparison of this so called 'permissive action' with what happens at the other extreme when an excess of the hormone is given. I wonder in how many instances we are working with definite types of phenomena whether in most of these instances a reaction is not merely on a curve beginning with a certain minimal requirement and then as the amount is increased proceeding to a greater and greater degree until an area is reached which we recognize as the excessive action of the hormone. Perhaps I might quote from some of Dr Ingle's work. As he has demonstrated there are instances in which an adrenalectomized animal that has been partially or totally depancreatized will show when given small quantities of adrenal hormone and then placed in a particular circumstance an increase in glycosuria which obviously could not have occurred as a result of any increase in these hormones. And yet as we all know and as Dr Ingle has also shown when an animal is given an excess of any of these hormones the net result is also an increase in glycosuria. It may be worth while to discuss this interesting phenomenon further since it obviously might not only happen with an induced glycosuria but also in many other circumstances with which we have come to believe the adrenal hormones are concerned. Dr Ingle what are your present views on the problem?

great many deficiencies in which the action of a hormone is not apparent. One of the best examples is the effects of the growth hormone. As far as I know, if an animal is put in any situation in which the rate of catabolism is increased, nitrogen loss occurs, whether it is due to amino acid deficiency or any other deficiency. Under these circumstances, there probably will not be growth stimulation from administered growth hormone. Yet, if vitamin B deficiency is involved, adding the minimal amount of the vitamin will again allow the hormone to display its normal effects. The point you have raised with the adrenal cortical steroids seems to fall in this general class, that is, in the face of many types of deficiency, the normal effects of the hormone are not exhibited. I was particularly interested that giving the estrogen plus the adrenal cortical steroid is the same as giving a large excess of the adrenal cortical steroid.

Ingle: When cortisone and estrogen are combined, a marked glycosuria and hyperglycemia is induced in normal animals under conditions in which neither estrogen nor cortisone alone would cause glycosuria. (4) This may support the hypothesis that there is a continuous curve of sensitivity to cortical hormones.

We have studied related problems during the past year. Any sort of severe stress increases the secretory activity of the adrenal cortices, and we have been interested in the metabolic consequences of adrenal cortical activation during stress. Offhand, one would expect that any stress which causes adrenal cortical activation should cause exacerbation of diabetes, but we have found only one stressful agent which is diabetogenic. Exercise causes the glycosuria of the diabetic animal to decrease. Exposure of the diabetic rat to cold is followed by a very gradual fall in the level of glycosuria, and when exposure to cold is stopped, the glycosuria gradually rises to its prestress level. The slowness of these changes is surprising. Most toxic agents either have no significant effect upon glycosuria or they cause a slight decrease.

The one toxic agent which causes exacerbation of diabetes in the partially depancreatized, force-fed rat is ethylenediamine (5). For these studies, a very mildly diabetic rat, maintained on 4 to 5 ml of adrenal cortical extract per day following adrenalectomy, is used. Ethylenediamine in the adrenalectomized, depancreatized rat treated with a uniform intake of adrenal cortical extract did not result. We found, in an exacerbation of the diabetes. Several indirect lines of evidence would support the conclusion that the diabetogenic effect of ethylenediamine is mediated by the adrenal cortex, but ethylenediamine will exacerbate diabetes in the absence of the adrenal glands.

We have a hypothesis as to what happens. It is known that diabetes becomes less severe during states of adrenal cortical insufficiency. It is

Ingle The permissive action of the adrenal cortical hormones can be illustrated by the relationship of the diabetogenic effect of estrogen to the adrenal cortex in the rat (1). Since certain of the adrenal cortical hormones are diabetogenic and since large doses of estrogen are among the noxious stimuli which cause the adrenal cortices to enlarge it seemed probable that the exacerbation of experimental diabetes by estrogen was due to stimulation of the anterior pituitary adrenal cortex axis which produced a state of hypercorticalism. This hypothesis was supported by the observation that estrogen had little or no effect upon glycosuria in the adrenally insufficient, partially depancreatized rat. However, when such adrenalectomized rats were treated with a moderate uniform intake of adrenal cortical extract during the entire experiment the addition of estrogen caused a full blown exacerbation of the diabetes as evidenced by hyperglycemia and glycosuria.

I formerly thought of the permissive action of the adrenal cortical hormones as an all or none effect. More recently it has seemed probable as Dr. Long has suggested that there is a dose relationship to responsiveness at least for certain metabolic processes. Most of our studies are concerned with the role of the adrenal cortex in certain metabolic adjustments to stress. The hormones of the adrenal cortex play a permissive rather than a regulatory role in some and perhaps many adjustments to stress (2). Since the need for these hormones is greatly increased by severe stress it might be expected that increased amounts of hormones would be required to support a state of eucorticalism and consequently permissive action during severe as compared to a mild stress. We have data which seem to require such assumptions.

How the diabetogenic effect of adrenal cortical hormone overdosage relates to the permissive action of these same hormones in supporting the extra adrenal diabetogenic action of other agents remains a puzzling problem. The depancreatized animal becomes diabetic because it lacks insulin not because of altered adrenal cortical function yet when the adrenal glands are removed from the diabetic animal there is striking amelioration of the diabetes (3). The cortical hormones must be present in order to support the full blown diabetic state. This was one of the first observations which illustrated permissive action. In animals with a normal pancreas and supply of insulin the administration of an excess of the adrenal cortical hormones can also cause severe hyperglycemia and glycosuria. Should the diabetogenicity of the adrenal steroid hormones be regarded as a primary action of these compounds themselves or does hypercorticalism simply sensitize or release the inhibition of other physiologic mechanisms which then run wild and cause a diabetic state?

Long I had another question in mind also. There are probably a

Long That is the point I was interested in that there is really a curve beginning at a minimal dose below which nothing happens and continuing until with increased amounts more and more happens

Lukens In the case of the cat which has a very high requirement for cortical hormone it was a long while before we could find any hormone that would have a diabetogenic effect in that species The level for permissive action then differs with different species and within a certain range for each species Would that be a fair statement?

Ingle Yes

White The type of stress may also affect in each instance the degree of utilization of the steroid by tissues

Long Would you say in reference to the animals with diabetes that the glycosuria disappears because the supply of adrenal cortical hormone is not adequate to maintain it?

Ingle I suspect that muscle work and exposure to cold cause a decrease in the glycosuria of the diabetic rat because we have introduced a new factor namely an increased utilization of carbohydrate to meet increased energy needs At least some acceleration in glucose oxidation can take place without added insulin

Pitts What are you measuring so far as the total economy of the animal is concerned when you measure whether or not it puts out glucose in the urine? It seems to me that it is a very complicated end point and one that can be influenced in many different ways One way would be the effect of cortisone directly on the kidney Even that is complicated for glycosuria might be effected by changes in filtration rate cellular activity or a combination of both On the other hand are you studying metabolic effect say a diabetogenic action? Are you necessarily always studying the same thing? Might not under one circumstance the appearance of glucose in the urine be due to one cause and under the action of another agent might it not result from entirely different causes?

Ingle This is true We are confronted with the question of whether a state of hypercorticalism can exist in the diabetic organism without causing an increase in glycosuria We do not know that the level of glycosuria is a valid index of either hyper or hypo-corticalism However in our studies on permissive action we have employed several other criteria (2)

Ralli Are you leading us Dr Pitts to the role of the kidney in this situation?

Pitts Yes I think that in studying glycosuria the kidney cannot be disregarded but I readily admit that the kidney is not everything in glycosuria

Long In most cases when you have found glycosuria you have also found that the blood sugar was increased?

also known that adrenalectomized animals on a fixed intake of adrenal cortical hormones adequate to maintain a state of eucorticalism during nonstress conditions become adrenally insufficient during severe stress. It can then be expected that certain metabolic adjustments to severe stress which are dependent upon the permissive action of the cortical hormones will drop out because the limited amounts of cortical hormones are no longer adequate to support them. We consider it probable that ethylenediamine has an extra adrenal diabetogenic effect which is masked in the adrenalectomized depancreatized rat given a fixed moderate intake of exogenous hormone because the toxic action of ethylenediamine has caused a state of adrenal cortical insufficiency. Adrenal cortical insufficiency is averted in the ethylenediamine treated animal having an intact anterior pituitary-adrenal cortex axis, for the increased secretion of adrenal cortical hormones maintains a state of eucorticalism which is essential for permissive action.

The implications of this hypothesis are important. During severe stress it may not suffice merely to maintain a uniform intake of hormone in order to support permissive action. In the nonadrenalectomized animal the increased secretory response of the adrenal cortices may be required to support permissive action or in adrenalectomized animals an increase in exogenous hormone may be required to support responsiveness to extra adrenal stimuli.

The hypothesis has a bearing on the interpretation of Dr. Selye's concepts of the role of the adrenal cortical response to stressors in the etiology of the adaptation diseases. I hope that later in the Conference there will be an opportunity to discuss some of his experiments along these lines.

Lukens: When a rat is on a minimal life maintaining dose of adrenal extract is it then able to have diabetes?

Ingle: Not with a minimal life maintaining dose. The adrenalectomized rat will continue to live when treated with 0.25 to 0.5 ml of aqueous adrenal cortical extract per day. In order to restore responsiveness to a diabetogenic agent the requirement would be about 3 ml of the same extract.

Lukens: Or six times as much?

Ingle: Yes. That is about what the animal needs to restore the status quo of eucorticalism under nonstress conditions or mild stress. If the adrenalectomized animal is subjected to severe stress larger amounts of exogenous hormone are required to support or permit normal metabolic responsiveness.

Lukens: The permissive action then can take place within certain dosage limits?

Ingle: Yes.

Pitts I was thinking purely in terms of the thyroid response to variations in environmental temperature as being rather slow

Ingle Sellers (8) has shown that as far as metabolic response is concerned the thyroidectomized animal shows the response to cold

Lukens Have you ever produced increased glycosuria of a week's duration with any stimulus? I ask in reference to Dr Astwood's question because the length of time it takes for an infection to develop and to produce metabolic effects is such that it might well be missed in the ordinary acute laboratory experiment of one day's duration

Ingle Ethylenediamine is the only severe stressor which exacerbates diabetes in our limited experience. We have given it for 10 days. Estrogen, a mild stressor, has been given for 30 days

Astwood A number of people have studied the effect of infections in diabetic rats and as far as I know the result has always been that the diabetes has improved as measured by daily glucose excretion

Ralli Isn't that specific for the rat? The depancreatized dog, for example, responds differently from the rat to infection

Ingle Have such studies been done on animals given a uniform food intake? I would expect the infected animal to eat less

Astwood I think that is one of the difficulties

Long If a stitch abscess occurs following pancreatectomy or if an abscess develops in the peritoneum, the effect on the diabetes is much the same as in the human diabetic. In both the cat and the dog, incision and drainage of the abscess is followed by a prompt decrease in the severity of the diabetes. The rat, however, appears to be atypical

Lukens Rats are enormously resistant to many infections

White There is, I believe, a sort of unspoken indication among the discussants that one would generally expect the same kind of response to all types of stress, and I think this may relate to Dr Pitts' point that the glycosuria is really not only an end point but something measurable at the conclusion of a series of reactions. I am sure you would agree, Dr Ingle, that it would be very surprising if all of these stressors had the same relationship to permissive doses of adrenal cortical extract. Not only are different degrees of requirements imposed upon the tissues for the amount of adrenal cortical steroid which is needed to withstand the particular stress, which is the point I made before, but many of the stressors which you are using have differing effects on other metabolic processes, e.g., cold versus ethylenediamine. When the stressors are in the form of agents which not only have a relationship to gluconeogenesis and the end point being measured, glycosuria, but which also have effects on other processes, e.g., perhaps direct combination with enzyme proteins, it would seem to me very surprising if all these stressors were exerting the same influences and operating via the same

Ingle Yes It isn't just a matter of changing the renal threshold

Sayers May I ask whether the blood sugar is decreased in animals that are exercised?

Ingle Yes

Sayers What about the other stresses that you employed for instance cold or ethylenediamine?

Ingle We are studying the effect of ethylenediamine upon the level of blood glucose at the present time. These studies show that the increased excretion of glucose is accompanied by a rise in blood glucose during the administration of ethylenediamine.

Astwood I wonder whether we could induce Dr. Ingle to discuss the peculiar fact that in the human being an illness, particularly an infection, makes the diabetes worse while in a rat, as he has already mentioned, a stress seems to make the diabetes better.

Ingle We have not studied the effect of infections in the rat. Fractures which may cause a temporary exacerbation of diabetes in some patients cause a small increase in the glycosuria of the diabetic rat but only for a day or two. Nonspecific stressors do not have a uniform effect upon diabetes in the rat. Most stressors have no significant effect until the animal is brought near death. A few toxic agents such as dilute solutions of formaldehyde cause a significant suppression of glycosuria (6).

Pitts Have you tried the stress of increased thyroid activity brought about by the administration of thyroid hormone?

Ingle We have not studied the effect of thyroid hormone upon glycosuria. According to Houssay (7) the administration of thyroid causes some exacerbation of diabetes. We have shown that the effect of thyroxine upon nitrogen balance is independent of adrenal cortical function.

Glycosuria is not the only criterion that we have used in studies of permissive action. We have used hair growth, work performance and in several studies nitrogen balance as a criterion. The adrenalectomized rat treated with adrenal cortical extract shows a normal catabolic response when exposed to cold.

Pitts Why does it seem odd to you that the response to cold should be rather slow and that the return to normal should also be slow. It would appear to me an eminently reasonable thing.

Ingle I had expected that the change in glycosuria would be directly related to the energy requirement of the animal, falling abruptly when the animal was exposed to cold and rising abruptly when the animal was returned to room temperature. The response of the glycosuria level to periods of exercise and rest is very prompt whereas the response to and recovery from exposure to cold is slow. What is your basis for expecting the slow response to cold?

eosinopenia It is obvious that if these other substances are mobilized the overall eosinophil response will be enhanced

Loew Do I understand you to say that with epinephrine an eosinopenia can be produced after adrenalectomy?

Long Is that a matter of relative dose?

Thorn It can be done very easily with 0.5 to 0.5 mg of epinephrine

Long I thought that at one time you published several papers in which you said you did not get it with 0.25 milligrams

Thorn The majority of patients with Addison's disease did not get a significant eosinopenic response with 0.3 mg injected subcutaneously Epinephrine given relatively rapidly intravenously is an effective means of producing an eosinopenia but if the same dose i.e. 0.3 or 0.5 mg is spread over a period of four hours rather than given within a period of 30 minutes one may not observe an eosinopenia Thus the eosinopenia in a patient with or without his adrenals would appear to depend upon epinephrine attaining a threshold dosage

Long Isn't that contrary to the work of the Finnish investigators (9) who infused epinephrine over long periods and found an eosinopenia?

Thorn All I can say is that it appears to us that in the same individual a dose of epinephrine is more effective in regard to eosinopenic action if given more rapidly

Rall Has it anything to do with the blood flow?

Thorn No

Gellhorn I wonder whether the fact that epinephrine acts more effectively when given in a short time is an indication that a central action is involved whereas in the case of ACTH a peripheral action is involved

Thorn There is an interesting comparison between the two hormones In the case of epinephrine it appears to be a threshold discharge phenomenon whereas with ACTH time and a build up reaction is necessary to obtain a maximum response For instance one unit of ACTH given over a four hour period gives a small but definite increase in 17 hydroxysteroid excretion whereas one unit of ACTH given as a twenty four hour continuous intravenous infusion may cause a very marked degree of adrenal activation Regarding epinephrine no one to my knowledge has demonstrated by hormonal assay methods any activation of the adrenal cortex with epinephrine in man

Gellhorn I wonder whether I understood both Dr Pitts and Dr White correctly that the concept of nonspecific stress has been useful in the investigation of these problems involving the adrenal cortex? In a way I believe that the concept of nonspecific stress is a prescientific concept Specificities seem to be involved in every one of these stresses and what has been emphasized are certain common factors or common

mechanisms even though they affected the end point, glycosuria to the same degree

Ingle The only reason for expecting nonspecific stressors to affect glycosuria in the same manner arises from Dr Selye's emphasis upon the uniformity of the pattern of response to different kinds of stressors and emphasis upon the role of adrenal cortical activation in mediating the metabolic responses to stressors. We and others have found wide differences in some metabolic responses to stressors and have shown that the exciting mechanisms for some responses to stressors are extra-adrenal

Long If an animal is given a constant dose of adrenal cortical hormone and subjected to cold does his eosinophil count fall? This could be used instead of the glycosuria as an indication of adrenal cortical activity

Ingle We have not studied changes in eosinophils

Sayers Has an eosinopenia been observed in adrenalectomized animals on a constant dose of cortical hormone after cold exposure?

Long I was wondering whether anybody here had done this experiment. The eosinopenia obviously stems from the so called permissive action that is the lytic effect if it is a lytic effect on eosinophils which is usually said to be due to a rising content of adrenal cortical hormone in the blood. If the amount of adrenal cortical hormone in the blood is kept constant by injection and then a stressing agent is applied what happens to the eosinophils? Does the eosinophil count provide a better index of the effects of stress than glycosuria? Will it always respond the same way?

Ingle There are certain stimuli which will cause eosinopenia in adrenalectomized patients and patients with Addison's disease but I read a manuscript the other day in which the author insisted that this happens only when functional cortical tissue remains

Thorn I would take exception to that

Ingle Many people agree with you

Thorn If a given patient fails to respond to an infusion of ACTH which is the case in patients with Addison's disease and patients in whom we have carried out bilateral complete adrenalectomy and if in the same individual an eosinopenia can be easily induced by administering 0.3 to 0.5 mg. of epinephrine it does not seem plausible to account for the eosinopenia on the basis of adrenal steroid mediation. On the other hand the most potent stimulus for eosinopenia in the presence of adequate adrenal cortex is the exogenous administration of ACTH. In view of this I feel that we must assume that there are other substances and other mechanisms in the body which independently of the adrenal cortex or adrenal cortical steroids are capable of producing an

tomized In this respect the experimental animal differs considerably from our studies on intact man

Long That is not what you said before You said there was no evidence

Thorn There is no direct evidence to date in man of adrenal cortical activation with epinephrine

Lukens If the tissues use up all the excess adrenal hormone which is put out it is the same thing

White No it is not the same thing

Lukens Correct but it might have the same outward appearance to one measuring urinary steroids

Vogt In man it is difficult to keep up what appears to be an increased output of cortical hormone due to epinephrine for any length of time That may possibly explain why there is not increased excretion let us say of metabolites or even cortical hormone in the urine Man is more difficult to study in this regard

Thorn All of our experiments have been carried out with four hour fractionations and occasionally with two hour fractionations of the urine Thus I doubt whether we would miss a temporary increase in secretory activity Certainly the eosinophil count is down at the time that the urinary assay is carried out

Vogt But certainly the eosinophils take time to go down don't they?

Thorn It is true that the eosinophils do take time to achieve their maximum fall but we have shown that when a fall of 50 per cent is attained with ACTH an increase in urinary steroids can be detected during that period very easily

Vogt But much of the cortical hormone must be being wasted

Thorn No there is not much excess of hormone in the range of eosinophil fall between 40 and 80 per cent It is only over a 90 per cent fall in eosinophils that we would feel that an excess of hormone might be secreted which would not necessarily be reflected in the eosinophil fall but as a greatly increased urinary excretion of steroid

Vogt How do you administer the hormone?

Thorn The cortical hormone in these experiments is always given intravenously We usually employ a constant intravenous infusion

Vogt When you give it by single injection certainly a lot is wasted by simple spilling over into the urine

Thorn With a sudden large intravenous infusion of hormone of course there would be an excess available and there might be appreciable spilling over into the urine It would seem to me that studies in intact man in which the hormone was given either as a sudden single intravenous injection or as a continued sustained intravenous injection would match either of the most likely possibilities which would occur when the gland itself was stimulated

effects. Nevertheless, every stress group may still involve specific differences.

Pitts I had in mind the fact that cold and exercise might be interpreted physiologically as having an effect, say, on glucose utilization and would lower blood glucose that way. Concerning other stresses, I have no idea what the effects on glucose utilization, glycogenolysis, or gluconeogenesis might be or on tubular reabsorption or on any of the other possible mediating factors. There might be an element of specificity in some of these stresses, and yet all of them might lead to the same general end in different ways. Or they might lead to entirely different ends. I don't think they are as nonspecific as implied.

Long Getting back to Dr. Thorn's remark that there has not been any demonstration that epinephrine stimulated the adrenal cortex, is not the fall in eosinophils in a man given epinephrine indicative of secretion of adrenal cortical hormone?

Thorn As my previous discussion indicated, an eosinopenia following epinephrine is not in itself conclusive evidence of adrenal cortical steroid discharge.

Long Putting aside for the moment that a prolonged infusion of epinephrine will not alter the eosinophil count and taking the doses that you first proposed in man, 0.25 mg. given to a normal individual which was followed in four hours by a 80 or 90 per cent drop in eosinophils, was that not specific and had that nothing to do with the stimulation of the adrenal cortex?

Thorn Three laboratories working independently have been unable to demonstrate that epinephrine administered to man stimulates the adrenal cortex to increase its output of adrenal steroid, as measured in one laboratory by blood hormonal levels and in two laboratories by urinary excretion of hormones. From these data I think that at present we are not justified in assuming that the eosinophil fall following epinephrine necessarily parallels adrenal cortical activation. It would appear to be a completely independent phenomenon.

Long This demonstrates then that man is very different from the dog. I don't have to speak for Dr. Vogt, but certainly her experiments clearly demonstrated that if epinephrine is given in the physiologic range, certainly within a few minutes there is a marked increase in the output of adrenal cortical steroids. What you are saying in effect is that the human pituitary system does not respond to epinephrine.

Thorn All I can say is that in man we have been disappointed in not being able to confirm the observations made in animals. It is to be noted, however, that in Dr. Vogt's experiments, as she has explained, epinephrine did not lead to an increase in output of adrenal cortical hormone from the adrenal gland until the animal was splanchnic

that in man at least epinephrine is a potent mechanism for producing the change in these circulating cells

Rall Do you feel that the uric acid creatinine ratio gives any objective evidence of adrenal stimulation?

Thorn In short term experiments I feel that changes in urinary uric acid and creatinine ratio are of very limited value in the interpretation of adrenal cortical response

Vogt If cortical hormone or ACTH is injected into a patient who is not exposed to stress and therefore so to speak doesn't need the extra dose there are signs of excretion in the urine. Do you think that this means that when the same person produces cortical hormone in response to stress the same amount will appear in the urine? May it not be metabolized differently and therefore not be found?

Thorn Such experiments are now in progress. We have demonstrated the fact that an increase in urinary steroid excretion can be reliably shown following the continuous intravenous administration over a period of four hours of one to two units of ACTH i.e. 0.25 to 0.50 units per hour. The experimental design which we are employing is to test the individual's sensitivity to this dose of ACTH study the individual's response to exposure to cold for a four hour period and then add ACTH to the exposure to cold to detect whether the ACTH stimulation under these circumstances gives rise to the added quantity of urinary hormone which we are able to detect in the absence of the cold.

Vogt Would it not be clearer if instead of using 0.25 units of ACTH you were using a small dose of cortical hormone itself?

Thorn No. If one is using intact individuals ACTH I believe is preferable at this dose level. The spontaneous involution of the adrenal which occurs with 11-17 oxygenated steroid administration makes the interpretation of urinary changes unreliable in individuals with intact adrenals given small doses of steroid. This of course does not obtain when massive hormone dosage is employed.

Long So many papers are written nowadays in which the criterion of lack of or increased activity of the adrenal cortex is based on eosinophil counts. I don't need to remind this group that when epinephrine is given or when an animal is subjected to stress which causes it to release epinephrine one of the things that happens is splenic contraction. Certainly in man as the Finnish investigators and many others have shown the first effect of epinephrine may be a 100 per cent increase in the eosinophil count. Thus there is a high degree of uncertainty as to the extent of the initial rise even before the eosinophil count begins to fall so that under some circumstances when a second measurement of eosinophils is made it is found to be back at the original level and that is interpreted as a lack of adrenal cortical activity.

Vogt According to Colfer de Groot and Harris (10), intravenously administered epinephrine does not cause a fall in peripheral lymphocytes which is a similar type of test. If Dr. Thorn's interpretation of his observations in man is right, it may be that man behaves like the rabbit and not like the dog.

To come back to the question of the possibility of using in animal experiments, the determination of the eosinophils in the same way as Dr. Ingle uses the glycosuria, there is one difficulty at least where mice are concerned. Apart from epinephrine, with which I have had no experience, there are certain substances which will cause a fall in eosinophils in adrenalectomized mice. For example, very small quantities of dog's plasma globulin will cause a fall in eosinophils in the completely adrenalectomized mouse. Therefore, there must be at least two mechanisms which can cause in the mouse a fall in eosinophils, one being the release of cortical hormone and the second some mechanism which can be stimulated, for instance, by the injection of dog globulin.

Thorn I believe that the fall in circulating eosinophils correlates extremely well with adrenal cortical hormone output following exogenous ACTH stimulation. However, it is evident that since factors other than the adrenal cortex may induce the eosinopenia noted in stress, it is of limited significance in terms of interpreting the participation of the adrenal cortex. On the other hand, a failure to observe an eosinopenia is a considerable help clinically as it suggests the absence of significant adrenal cortical activation at that time. Thus, it would appear that in evaluating the capacity of the adrenal to respond to exogenous ACTH, the eosinophil response is extremely helpful, whereas the overall eosinophil change in the reaction to stress does not necessarily prove to be a true indicator of adrenal cortical participation.

Rall Are you suggesting that if an individual is exposed to cold stress, an eosinopenia will be provoked? Short term exposure to cold is notably unpredictable in its effect on the excretion of the cortical steroid, and I believe that in man consistent changes in the steroid excretion do not occur.

Thorn I agree with that.

Rall You can't say that that doesn't prove the adrenal is not stimulated.

Thorn That is correct, but one cannot say that the adrenal has been stimulated if one is not able to demonstrate either an increase in circulating hormone level or the excretion of an increased quantity of urinary steroids. Although it is undoubtedly true that exposure to cold increases the adrenal cortical activity, one is not justified in stating this categorically on the basis of an eosinopenia, since we have demonstrated

brain extracts also cause a fall and that this is due to their springomyelin content

Thorn I believe Dr David Hume showed that using specially prepared hypothalamic extracts

Long Yes The point I am trying to get around to is that using any brain extract not necessarily from the hypothalamus would do it

White In your experiments with epinephrine Dr Thorn in the human have you followed lymphocytes? Does one get a lymphopenia in the adrenalectomized patient with epinephrine?

Thorn It is more difficult to measure significant changes in lymphocytes in man but the direction of change is similar to that of eosinophils It is our interpretation that this is due to the much more rapid turnover of lymphocytes and the larger reservoir available

White Certainly in the mouse the lymphopenia with epinephrine does not occur if the animal is adrenalectomized

Thorn I cannot give any figures on this in man

White We are focusing a good deal of attention perhaps too much attention on a common specific pathway for stresses via the adrenal cortex I wonder whether a true measure of what stress does physiologically isn't better reflected in the adrenalectomized animal?

Pitts Dr Ingle as a point of information is there such a thing as nonspecific stress in an adrenalectomized animal? Is the nonspecificity casually related to the response of the adrenal cortex?

Ingle In Dr Selye's concept of the alarm reaction he has emphasized the similarity of the pattern of certain biochemical physiologic and morphologic changes which ensue irrespective of the nature of the stressor The adrenal cortex is considered to be activated during exposure to any severe stressor and has been considered by Dr Selye and many others to mediate some of the biologic consequences of exposure to stressors There is no doubt about the nonspecificity of noxious stimuli which will elicit the alarm reaction but perhaps the similarity of responses to different stimuli and the role of the adrenal cortex in mediating the various responses making up the alarm reaction have been overemphasized

White One piece of evidence that stressors do different things and that they don't all act via the adrenal cortex is seen in the extent and magnitude of the lymphocytosis which can be produced in an adrenalectomized animal by varying kinds of stress The striking effect is one of tremendous lymphocyte production in the absence of the restraining influence of the adrenal cortex and yet the degree to which various stressors do this will differ quite markedly from one to another What the mediating mechanism is I am not certain but just as there is a difference in the rate of glucose utilization depending on the type of

I don't think one is entitled to say that either. In our splenectomized rats the eosinophil count begins to fall soon after epinephrine injection but in the intact rat, there is initially a large rise.

Thorn: That is true for epinephrine. It certainly would not be true for the administration of crystalline steroid as there one observes a continuous fall in eosinophil level.

Long: Yes. I am speaking of those circumstances cold and so on in which there is a release of epinephrine and autonomic discharge.

Thorn: To summarize our experience the absence of eosinopenia following an initial exposure to a stress situation is strong indirect evidence against adrenal activation. On the other hand one is not justified in attempting to interpret quantitatively adrenal cortical response to stress on the basis of the eosinopenia alone since this reaction may be induced by factors other than the adrenal steroids liberated during the exposure to stress.

Long: I would not disagree with that. It has already been said in other places that there are uncertainties regarding the eosinophil count.

Thorn: I don't believe it is a question of uncertainty regarding the eosinophil count but a question of capacity to interpret the changes which are measured.

Bush: It is surely no more logical to say that the absence of eosinopenia is definitely correlated with the absence of adrenal cortical stimulation in the light of present knowledge than it is to say the reverse. Until we have investigated whether there are any agents which can cause a rise in eosinophils we cannot assume that the absence of a fall indicates an absence of adrenal cortical stimulation.

Loew: How did you get to the idea to check globulin?

Vogt: In work carried out by Dr. Bibile and myself we were using eosinophil counts in adrenalectomized mice or trying to use them in order to test for cortical hormones in blood. We ran up against a difficulty in using dogs' blood in that quite small quantities of arterial dog blood contained something which caused a fall in the eosinophils. From a quantitative point of view it was impossible that this effect was due to cortical hormones because it would have meant that there were incredible quantities of cortical hormones in the arterial blood. We started fractionations and found the globulin fraction active. We haven't analyzed the effect further.

Loew: You did not use any other globulin?

Vogt: We have tried a few different species. It was only the dog which produced it regularly or gave rather large falls. Occasionally it happened with the cat and the human but mouse, rat, guinea pig and fowl plasma did not cause it.

Long: I have heard that somebody in Great Britain has shown that

Li What is the exact dose?

Vogt The dose corresponds to 0.1 ml plasma. After it is fractionated, I don't know what it corresponds to but it is not very much just the globulin contained in 0.1 ml dog plasma. It would have to be a very potent steroid if the amount contained in the globulin fraction of 0.1 ml plasma suffices. It might of course be any substance associated or precipitated with the globulin but certainly it cannot be extracted with an organic solvent as can ordinary steroids. That is all I can tell. It may be a steroid which is unextractable.

Rall Does this response occur with plasma from any number of dogs?

Vogt There is a slight variation in the intensity. A particular plasma has the same activity on different batches of mice but different dogs' plasma have a different degree of activity. Plasma from one animal may be potent in a smaller dose than the plasma from another animal. There is variation from dog to dog.

Rall Does it have any relation to species?

Vogt That I don't know.

Lukens Does it have any relation to the preceding stimulus of the dog?

Vogt Well, the blood was collected either by venipuncture in the conscious dog or by arterial bleeding under anesthesia. We did not try to correlate the effects with the conditions under which the blood was obtained.

Thorn Favour has shown a very good *in vitro* lympholysis with human plasma. Eosinopenia cannot be demonstrated *in vitro* but a lympholysis can. However, it never seemed to me that the amounts of plasma involved were large enough to account for that effect on the basis of the steroid hormone although one cannot say for sure.

Vogt If globulin is prepared from plasma by dialysis, the dialysate is the ineffective part and what is left behind is active. If it is a steroid of some sort, it must be very firmly associated with the protein. It is not dialyzable or extractable with an organic solvent.

Bush Isn't it unlikely that it is a steroid because it is inactive in the cold exposure test?

Vogt Well, it is certainly not an adrenal steroid but there may be something else which acts on eosinophils.

Bush You mean it might be a trace of cortisone or something else?

Rall Could this be an allergic reaction of some kind?

Vogt If it is, it could be only one of those inborn allergic reactions for which preceding sensitization is not necessary. That is of course quite possible. That is why Dr. Bibile, who did these experiments, tried all sorts of things hoping to find something else which would produce

stress so there could very well be a difference in the rate at which lymphocytes are utilized

Long Dr Pitts and Dr Ingle's last remarks lead to another point which is in line with what we have been talking about that is the emphasis which has been placed on the release of ACTH from the pituitary in response to stress. At times, the literature seems to indicate that this is the only thing which happens but is this true? When the animal is placed in the cold as Dr Pitts has pointed out there is also thyroid activation. We have very little information as to how rapidly that occurs but there is some work to indicate that it may be almost as fast as the response of the adrenal cortex. I believe that trying to assess the response to cold or to any of these stressful circumstances merely in terms of the adrenal cortex is probably misleading indeed because we know that in many circumstances ultimately the other endocrine glands are involved. In some circumstances, there is gonad atrophy in others, hypertrophy of the thyroid along with hypertrophy of the adrenal.

Li Is it possible also that there are different forms of ACTH and that different stresses might cause the secretion of different types of ACTH? For the past two months we have been trying out the effects of different preparations of ACTH on normal and hypophysectomized rats with respect to eosinopenia. We have one preparation which has a very marked adrenal ascorbic acid depleting activity as well as adrenal weight increasing potency but which produces no eosinopenia when it is injected into hypophysectomized or normal rats. There was found no correlation between the eosinopenic activity and the adrenal ascorbic acid depleting activity among various fractions. In fact reduction of the adrenal ascorbic acid depleting activity by NaOH heat treatment does not inactivate the eosinopenic activity which suggests that eosinopenic activity might be a separate factor.

Long How many kinds of ACTH would that make?

Li We don't know exactly how many different ACTH's there are. I was merely talking about ACTH activities.

I should like to ask Dr Vogt about the globulin. A possible explanation might be that there are adrenal steroids associated with the globulin which is causing the eosinopenia in the adrenalectomized mouse. Or have you tried using different kinds of protein?

Vogt Yes we have tried a number of proteins several albumins including ovalbumin and they were all ineffective. I think it is practically impossible that there are steroids in the globulin fractions. At least if there are they are not extractable with any organic solvent. It must be a very queer sort of combination and they would have to be there in very large quantities because the total amount of globulin required is extremely small.

MECHANISMS THROUGH WHICH THE ADRENAL CORTEX PRODUCES QUALITATIVELY DIFFERENT EFFECTS*

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I HAVE NOT ENTITLED my opening remarks "The plurality of adrenal hormones" particularly since Dr. Vogt is to speak about new work on the so called natural mineralo corticoid or salt active hormone. I merely plan to survey the evidence which suggests that the adrenal cortex can produce qualitatively distinct effects quite irrespective of whether different hormonal substances are produced.

In order to outline the ways through which the adrenal cortex might produce qualitatively different syndromes, may I first present in Figure 1 the now familiar sketch on endocrine correlations during stress. Before discussing the interrelations depicted in Figure 1, I should like to define my views concerning three points, because these are often misinterpreted in the current literature and hence have given rise to much confusion.

STRESS IS BY DEFINITION ALWAYS NONSPECIFIC

1) In earlier writings (1, 2) I defined stress as "the sum of all non-specific biologic phenomena (including damage and defense)". It may be localized (as exemplified by inflammation) or systemic (as exemplified by the general adaptation syndrome). Those who speak of various kinds of stresses evidently do not use the term according to this definition, yet I am not aware of any alternative definition.

A stressor is an agent capable of producing stress. I distinguish the systemic stressor or alarm stimulus (an agent capable of producing a general adaptation syndrome or G.A.S.) from the topical stressor (an irritant which causes local nonspecific tissue changes, e.g. inflammation or necrosis). Naturally, infections, cold, drugs, etc. produce qualitatively different effects because, apart from acting as stressors, they also possess specific properties of their own. The latter are super-

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the same reaction Horse serum and an extract of Ascanis and so on were tried but nothing had the same results

Long I still have a long list of questions here but I think in the interest of our conversations tomorrow we shall stop at this point

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THE SO CALLED PERMISSIVE ACTIONS OF CORTICOIDS ARE
BUT ONE OF THE PHENOMENA OF CONDITIONING

3) Not only hormones but stress dietary constituents and many other agents can act as conditioning factors. These have been defined as factors which have little or no activity in themselves but can significantly alter a response to a stimulus. For instance sodium can act as a conditioning factor of DCA activity stress which in the absence of the adrenals manifests itself by hypoglycemia can so condition the actions of gluco-corticoids that even virtually inactive threshold doses of these will produce marked hyperglycemia.

It is largely a matter of one's point of view whether in any such instance the hormonal or the nonhormonal agent is designated as the conditioning factor. It would be equally correct to say that the DCA effects are conditioned by sodium or that the sodium effects are conditioned by DCA. The important point is that when corticoids are administered or endogenously produced at ineffective levels they may nevertheless produce manifest effects by conditioning the body to certain stress responses. For example they may reverse hypo- into hyperglycemia. It is for this particular instance that Dr Ingle if I understand him correctly proposes the term *permissive action*. This strikes me as entirely acceptable since here the hormone does not so much produce an effect of itself as it conditions the body's response in such a manner that the effect of another agent is permitted.

To return to a discussion of Figure 1 although each stressor (cold infections nervous strain) has a variety of specific actions of its own which may condition the response of individual target organs during stress these specific actions are not illustrated. The drawing does show however that the stressor acts upon the target the body or some part of it directly (thick arrow) and indirectly through the pituitary and adrenal. Through some unknown pathway (question mark) a stimulus travels from the directly injured target area to the anterior pituitary. It notifies the latter that a condition of stress exists thus inducing it to discharge ACTH. It is quite possible that this first mediator of hormonal defense is not always the same. In some instances it may be an epinephrine discharge in others a liberation of histamine like toxic tissue metabolites a nervous impulse or even a sudden deficiency in some vitally important body constituent such as glucose or an enzyme.

ACTH stimulates the adrenal cortex to discharge corticoids. Some of these the mineralo-corticoids or prothelagic corticoids (PC) stimulate the proliferative ability and reactivity of connective tissue. By enhancing the inflammatory potential they help to put up a strong barricade of connective tissue through which the body is protected.

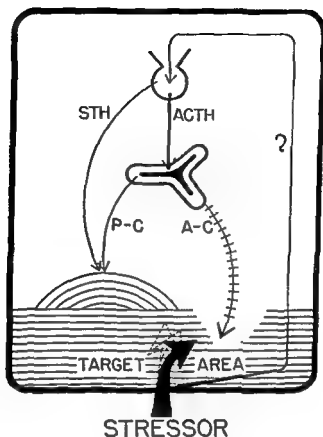


FIGURE 1 Schematic outline of interrelations between the stressor and the hormonal defense system

imposed upon their stressor actions and may condition the body's response modifying certain manifestations of the basically stereotyped and always nonspecific stress reaction

STRESS DEFINITELY OCCURS EVEN IN THE ABSENCE OF THE HYPOPHYSIS AND/OR THE ADRENAL CORTEX

2) Indeed some of our earliest experiments on the adaptation syndrome were designed to demonstrate that certain characteristic manifestations of stress (e.g. hypoglycemia hypochloremia) are rarely evident except in hypophysectomized or adrenalectomized animals in which the normal adaptive response of the hypophysis adrenal system is eliminated

transformations of a single secretory product into qualitatively different hormones must also be considered

Our latest experiments suggest that even the distribution of circulating corticoids in various organs or their selective fixation in certain tissues may result in qualitatively different effects. It is obvious that if for some reason an organ has a selective affinity for a corticoid this hormone can act on such an organ selectively even without any change in blood level or urinary titer. This may be a far fetched possibility at the present time since such selective hormone fixation has not yet been demonstrated to occur for endogenously secreted corticoids. It has however been shown that estrogens and thyroxin can be selectively concentrated in certain tissues. Furthermore experiments have demonstrated that when artificial fixation of hormones in the skin is achieved by a special experimental technique the local response is greatly exaggerated.

This theoretical introduction is somewhat lengthy and complicated. However I believe it is indispensable to formulate the problems clearly before attempting to interpret factual observations concerning the plurality of the mechanisms through which corticoids may exert qualitatively different actions.

Figure 2 illustrates the organs of two rats which have been treated with exactly the same amount of desoxycorticosterone acetate namely 10 mg per day subcutaneously during a period of 53 days. The organs on the left show manifest signs of the so-called DCA encephalopathy (with brain edema) cardiac hypertrophy enlargement of the kidney (with nephrosclerosis) and periarteritis nodosa of the mesenteric vessels. Most of the animals in this group died as a result of hypertensive changes. The organs on the right come from a rat which was treated with exactly the same amount of DCA and yet none of the typical changes are manifest. The brain heart kidney and the mesenteric vessels are macroscopically of normal appearance and this was confirmed by microscopic examination. All the animals in both these groups were specifically sensitized to the toxic manifestations of DCA overdosage by unilateral nephrectomy and by a 1 per cent NaCl solution given as drinking water. The protection of the animals represented on the right side of the picture was accomplished solely by hypophysectomy. In other words ablation of the pituitary appears to render the rat resistant to the typical hypertensive and rheumatic like changes induced by DCA. This protection is evident despite sensitization by such conditioning factors as unilateral nephrectomy and a high sodium intake.

On the basis of Figure 1 one would be tempted to assume that the toxicity of the prothogistic corticoid in this case DCA depended largely upon a pituitary factor perhaps STH. Here again in a certain

against further invasion by the pathogenic stressor agent. However, under ordinary conditions, ACTH stimulates the adrenal cortex much more effectively to secrete the so called glucocorticoids or antiphlogistic corticoids (A C). These inhibit the ability of the body to put up granulomatous barricades in the path of the invader; in fact, they tend to cause involution of connective tissue with a pronounced depression of the inflammatory potential. Thus they open the way to the spreading of infection.

As far as is known, ACTH always stimulates the adrenal cortex to produce the various corticoids in the same proportion and always with a great predominance of A Cs. However, the somatotrophic hormone (STH) of the pituitary also increases the inflammatory potential of connective tissue somewhat as the P Cs do, hence, it sensitizes the target area to the actions of the latter.

It is possible that the hypophysis also secretes some special corticotrophin which induces the adrenal to elaborate predominantly P Cs; indeed, STH itself may possess such effects, although this has not yet been proven. In any event, even if ACTH were the only corticotrophin, the actions of the corticoids produced under its influence could still be vastly different depending upon conditioning factors such as STH, which specifically sensitizes the target area for one or the other type of corticoid action. Actually, perhaps conditioning factors could even alter the response to ACTH of the adrenal cortex itself, so that its cells would produce more A Cs or P Cs. Thus, during stress, one or the other type of effect could predominate.

The fundamental reaction pattern to topical stressors is inflammation; to systemic stressors, the general adaptation syndrome. Various combinations of these two basic responses constitute the essence of most diseases.

Qualitatively different corticoid effects may arise during stress due to differences in the conditioning of the response at various levels, particularly in the target area, the hypothalamo-pituitary system and the adrenal cortex. For instance, an agent may, in addition to its stressor effect, have certain specific properties which alter the response of the target area directly. The hypophysis may respond during stress by the production of STH and ACTH in different proportions; indeed, it may produce qualitatively different corticotrophins, although this has not yet been definitely proved. The adrenal may respond to the same ACTH by the secretion of the various corticoids in different proportions, due to factors conditioning its own response to ACTH. The target organ response to any one corticoid may be altered by other corticoids, by STH, by sodium, and by many other factors. In connection with a plurality of actions arising at this last peripheral level, it is well to remember that metabolic

sense we might speak of an indispensable hypophyseal conditioning for the toxic DCA actions

The fact that such lesions cannot be produced in hypophysectomized rats by DCA has been shown before (3) and yet it could not be concluded that the pituitary was necessary for all the actions of this hormone*. Among the other usual DCA effects the polyuria was actually aggravated the hypochloremia was abolished while the enlargement of the kidney seen in intact DCA treated rats was merely diminished not abolished by ablation of the hypophysis. Apparently hypophysectomy influences the various effects of this steroid in different ways leaving some uninfluenced increasing others and abolishing yet others. This is what I have called selective conditioning of various targets

Recently my associate Dr Ernesto Salgado and I repeated and confirmed this work on a large number of hypophysectomized rats. In this series we observed furthermore that the pressor effect of DCA was only slightly reduced by hypophysectomy although the above mentioned cardiovascular and renal changes were virtually completely blocked. We also demonstrated that even simultaneous treatment with DCA and cortisone which in our experience is particularly damaging to the kidney of intact rats caused no nephrosclerosis after hypophysectomy (5). I mention these experiments on hypophysectomized animals to illustrate how the total picture of DCA intoxication can be qualitatively altered by the absence of hypophyseal hormones

Figure 3 illustrates another type of selective conditioning. On the left is the heart kidney and adrenal of a normal female control rat. On the right are the corresponding organs of a rat of the same size and age. This animal received heavy doses (20 mg twice daily) of lyophilized anterior pituitary (LAP) and of DCA (5 mg daily) seven months prior to autopsy during a period of twelve days. Both animals originally weighed 106 grams and both were sensitized to mineralo-corticoid actions by unilateral nephrectomy and a 1 per cent NaCl solution as drinking fluid. Twelve rats were subjected to the transient hormone overdosage. Upon cessation of both hormone and NaCl administration they appeared to be in perfect health yet all but this one animal died during the ensuing seven months with marked nephrosclerosis hypertension periarteritis nodosa and cardiac nodule formation. The adrenals were essentially normal. This last survivor also shows cardiac and aortal hypertrophy fibrous nodules in the heart (note the white

Attention is called here to a misprint on page 513 in the first printing of STRESS (4). The line referring to these DCA experiments (3) reads "typical hyaline nodules can be produced by this mineralo-corticoid compound in the absence of the pituitary" it should have read "typical hyaline nodules cannot be produced".

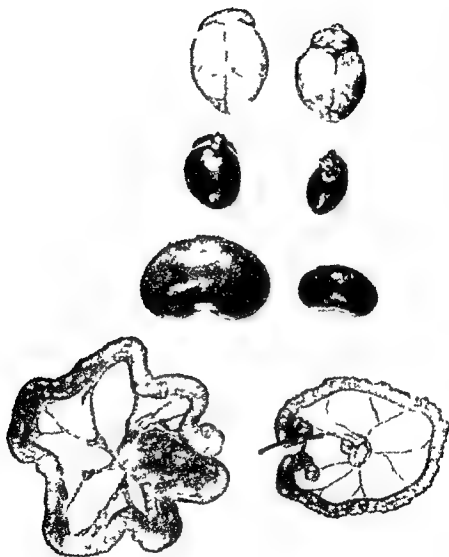


FIGURE 2 Inhibition of certain mineralocorticoid overdosage effects by hypophysectomy. Reprinted by permission from Selye H. and Horava A. *Second Annual Report on Stress*. Montreal ACTA Inc. 1952.

metacorticoid hypertension may be an appropriate designation for this phenomenon in analogy with the expression metahypophyseal diabetes used by Professor Houssay for that type of glycosuria which develops after interruption of pituitary extract treatment

In an informative systematic study of what he has called post DCA hypertension Green *et al* (8) called attention to the most important fact that this type of hypertension exhibits a striking similarity to the essential hypertension occurring in man

From the point of view of selective conditioning our observations are of special interest since they show that the time factor in itself can dissociate the usual manifestations of DCA intoxication and thus qualitatively alter the total picture of corticoid overdosage. During the metacorticoid hypertension the characteristic rise in blood pressure the cardiovascular changes and the renal changes typical of DCA overdosage are evident but other manifestations for instance the compensatory atrophy of the adrenal cortex are absent

Ingle Does the animal receive the high sodium load throughout the time it survives?

Selye In this particular group the sodium treatment was interrupted for a few weeks given again for a few weeks and so on just to show that hypertension and polyuria are aggravated in an animal sensitized by temporary DCA and LAP overdosage by a high sodium intake. I believe Dr Green found that sodium does not aggravate metacorticoid hypertension but the differences may be due to the fact that we used a slightly different experimental arrangement and in any case this is not relevant to the subject under discussion

Ralls Why did you unilaterally nephrectomize the animals?

Selye The experiment was primarily designed to show the briefest possible period of heavy hormone overdosage that could cause meta hormonal hypertensive changes at a later time. Unilateral nephrectomy and a high sodium intake are not indispensable for the production of such changes but these conditioning factors greatly facilitate their development

L These animals were not hypophysectomized?

Selye That is right

L Did you examine the pituitary in these animals?

Selye With ordinary hematoxylin eosin sections nothing noteworthy was seen

Astwood The animal at the right in Figure 3 shows the state at the end of 12 days?

Selye No That is seven months after commencement of the treatment. Both animals were unilaterally nephrectomized at the same time and when one got salt the other also got salt. The only difference

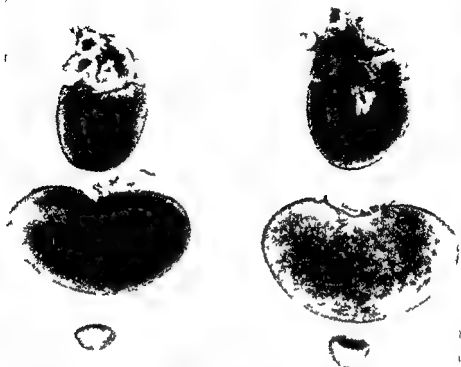


FIGURE 3 Meta hormonal changes in the heart and kidney after temporary overdosage with DCA and LAP Reprinted by permission from Selje H First Annual Report on Stress Montreal ACTA Inc 1951

spots in the center of the ventricle) and a granular nephrosclerotic kidney. Throughout the entire length of observation all these animals invariably developed acute exacerbations of their hypertension when ever they were placed on 1 per cent NaCl for a few days. The experiment illustrates that a brief temporary excess of adaptive hormones can cause a progressive and eventually fatal disease although at the time of manifest illness there need no longer be any hypophyseal or corticoid hormone excess (1).

Some former members of our laboratory Professor Sidney Friedman (6, 7) in Vancouver B.C. and Dr. Leal Prado* in Sao Paulo Brazil had previously and independently observed that even temporary overdosage with DCA alone may cause a hypertension which persists after interruption of hormone therapy. Hormone treatment must be continued much longer however to produce a lasting change. The term

*Prado J. L. *Estudos sobre hipertensao hormonal experimental*. These apresentada a Escola Paulista de Medicina, Sao Paulo (1950).

between the two is that the one on the left (as did all animals in this group) remained otherwise untreated while that on the right received hormone treatment during the first 12 days

Vogt Did they all react the same way in that group?

Selye Yes In the group given hormone treatment 12 out of 12 died and all of them showed lesions There was scatter only in the sense that not all the animals died at the same time

Figure 4 illustrates an experiment which was made possible through the help of Dr Astwood who supplied us with a sample of his oxycellulose purified ACTH At the top is the adrenal and kidney of an untreated control rat Neither this nor the two other animals illustrated were sensitized by partial nephrectomy or by a high sodium diet In the middle are the adrenal and the kidney of a rat which received Dr Astwood's oxycellulose ACTH preparation over a period of 25 days subcutaneously twice daily in oil The daily dose level was initially 0.2 mg and was then gradually raised in the course of treatment to 0.4 mg and finally to 0.6 mg During this time the rats did not grow because of the catabolic and growth inhibitory effect of ACTH Their adrenals became very large they lost their lipid granules and were extremely hyperemic Histologically there was intense dilatation of all the sinuoids in the cortex so that the organ assumed an almost spongy or cavernomatous appearance

Other animals of which a representative is illustrated by the organs in the bottom row received the ACTH treatment mentioned above but at the same time they were treated with an STH preparation kindly supplied by the Armour Company of Chicago The dose of STH was purposely always so adjusted as to maintain a normal growth rate despite the ACTH Consequently the animals in the third group were of the same size at the end of the experiment as the controls Despite this as can readily be seen the kidney in the rats treated with STH plus ACTH was considerably enlarged Histologically it showed signs of hypertrophy and hyperemia but no nephrosclerosis From a functional point of view it is also interesting to note that in this third group which received treatment with both pituitary hormones a pronounced polyuria developed

The most striking finding of course was the enlargement of the adrenal in all the rats of the third group The weight of the adrenal was above the maximum weight observed in any of the rats receiving ACTH alone It is tempting to accept such an experiment as definite proof of some corticotropic action of STH but to my mind the evidence is still not quite convincing It is quite possible that some metabolic change produced by the growth hormone outside of the adrenal so conditioned the gland that it responded differently to ACTH even if the STH itself had no effect on the adrenal at all



Fig. 11-1. Adrenal and kidneys of (a) normal control (top), (b) an ACTH treated (middle) and (c) adrenal glands ACTH and LSTH treated (bottom) rat.

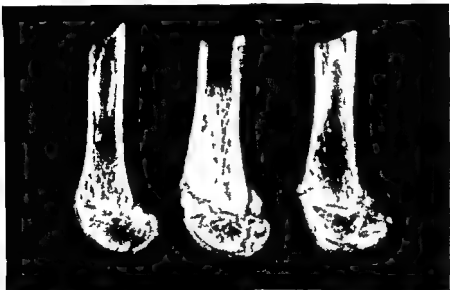


FIGURE 5 Effect of STH and stilbestrol given alone or in combination upon the bone of the rat. Reprinted by permission from Selye H. and Horava A. *Second Annual Report on Stress*. Montreal ACTA Inc. 1952

Having thus connected STH with adrenal function it may be of interest to discuss another experiment although it is not directly concerned with the plurality of adrenal hormones. Figure 5 illustrates the selective conditioning of STH by a folliculoid compound stilbestrol. I think it is quite proper to speak here of selective conditioning since not all the actions of STH are influenced by folliculoids and vice versa. Figure 5 shows sagittal cross sections through the lower extremity of the femur in three rats. On the left is the normal bone structure in an untreated control animal. In the middle there is a dense sclerotic femur in which much of the marrow has been replaced by trabecules of spongy bone. This animal was simultaneously treated with STH and stilbestrol. On the right there is a bone with very little sclerosis after treatment with stilbestrol alone. STH alone (not illustrated here) enlarges all parts of the femur proportionately without causing any sclerosis.

It was concluded from this experiment that although in the rat folliculoids cause very little bone sclerosis and STH causes none combined treatment with the two hormones results in a selective conditioning for this particular effect of the growth hormone so that the latter induces pronounced sclerosis of certain parts of the skeleton (9). Through what intimate mechanism of action the growth hormone is so modified in its effect upon the bone as to cause this sclerosis I would

Histologic examination showed that in most of the rats treated with both pituitary hormones there developed extensive necroses in the adrenals similar to those which were previously obtained with LAP (5) a preparation which contains both STH and ACTH. Indeed, in one of these animals an animal which died with extraordinarily large suprarenals there was actually no living adrenal tissue at all since histologically both adrenals had been replaced by necrotic tissue. This type of observation raises the possibility of a fatal adrenal insufficiency actually being induced by excessive corticotropic stimulation. Perhaps these necroses could be avoided at somewhat smaller doses of the pituitary hormones were given and the enlargement developed more gradually. Such experiments as well as studies on the effect of combined ACTH and STH treatment in hypophysectomized rats, are now being conducted by my associate Dr. Salgado.

Another interesting side observation made in the course of these experiments was that the so called brown fat which always surrounds the adrenal of the rat was very much stimulated by the combined treatment with STH and ACTH. These adrenals were suspended in a gelatinous mass of connective tissue containing brown fat cells which had assumed an appearance strikingly similar to that of stimulated adrenal cortical cells.

Li: How about the growth hormone alone?

Selye: Growth hormone alone also caused marked adrenal enlargement but only at dose levels which produced a considerable increase in somatic growth rate. Since this effect was not seen in hypophysectomized animals I am tempted to ascribe it either to the liberation of ACTH by the animal's own pituitary under the influence of STH or to some extrahypophyseal synergism between STH and endogenous corticotrophin.

Li: How about the histology?

Selye: Growth hormone alone, at the dose levels tested so far causes a fairly normal enlargement of the adrenal cortex that is one unaccompanied by necrosis, excessive hyperemia or excessive lipid loss. I might add that with one of Armour's growth hormone preparations which was virtually free from ACTH as judged by its effect upon the adrenals of hypophysectomized animals I have obtained adrenal weights up to 200 to 250 mg. in rats. In one case the adrenals weighed 394 mg. It must be said that this was seen only after several months of treatment the daily dose of STH being raised in proportion to the increased body weight.

Li: Is the adrenal cortex larger in terms of 100 grams of body weight?

Selye: Oh yes. I shall come back to this in connection with the conditioning of hormone effects.

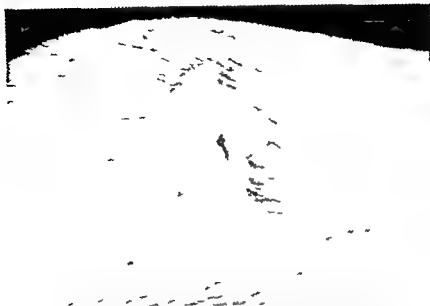


FIGURE 7 Closer view of the hydrocortisone deposit in an experiment similar to that illustrated by Figure 6. Reprinted by permission from Selye H. and Horava A. *Second Annual Report on Stress*. Montreal: ACTA Inc. 1952.

months before this picture was taken. Although histologically the skin structure remained essentially normal over the air space, hair growth was delayed, presumably due to some trophic disturbances. The hydrocortisone (1 mg of microcrystals) was deposited into the subcutaneous connective tissue the day before air insufflation, yet the size of the deposit hardly diminished in the following two months. Dilated veins are particularly visible in the atrophic skin area which covers and surrounds the crystals. Here all layers of the skin are extremely atrophic.

Figure 7 is a closer view of the hydrocortisone deposit in a similar experiment. Note particularly the complete inhibition of hair growth in the atrophic skin area which surrounds the hormone accumulation. It is noteworthy in connection with the problem of conditioning that a similar deposit of hydrocortisone acetate placed in the loose subcutaneous tissue of the same rat outside the pneumoderma has little if any effect on the skin except on the very limited area which immediately covers the crystals. Apparently the cutaneous dystrophy caused by the pneumoderma procedure so sensitized the skin to the action of this glucocorticoid that the limited hormone deposit was capable of affecting a comparatively large area of the surrounding skin. Qualitatively

not venture to say. It is my impression however on the basis of his tologic studies that folliculoids inhibit osteoclastic bone resorption while STH stimulates both bone formation and bone resorption. When both hormones are simultaneously given the latter effect of STH is blocked by stilbestrol, while its ability to stimulate osteogenesis persists. The result is a change which greatly resembles the experimental marble bone disease, as we produced it with parathyroid hormone many years ago. I should like to show some work which yielded techniques found to be especially informative in the study of certain corticoid actions. By the subcutaneous injection of 60 to 100 ml of air in the interscapular region of the rat it was possible to separate the dorsal skin from the underlying muscle and fascial layers. The skin thus detached remained perfectly viable but underwent a type of dystrophy which was selectively altered in response to various topically applied agents among others to corticoids. In analogy with the designations pneumoperitoneum or pneumothorax this might be referred to as a pneumoderma.

Figure 6 shows a pneumoderma with a hydrocortisone acetate deposit. Note the limited air sac on the back of this rat and the white hormone deposit. The hair was removed from the back with barium sulfide two



FIGURE 6. Pneumoderma with a hydrocortisone deposit. Reprinted by permission from Selye H. and Horava A. *Second Annual Report on Stress*. Montreal ACTA Inc. 1952.

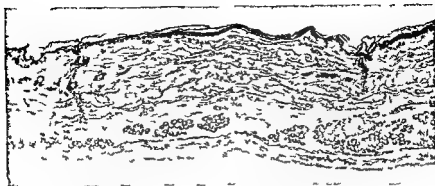


FIGURE 9 Microscopic appearance of the skin detached by the pneumoderma technique. This section was taken from the immediate vicinity of the hydrocortisone deposit.

especially this region which we examined by palpation at frequent intervals during the experiment. Particularly noteworthy is the fact that even the striated musculature underwent intense atrophy in the vicinity of the hormone deposit. Such muscle atrophy was not produced by the pneumoderma itself nor by a similar hydrocortisone deposit injected into ordinary, that is not detached skin. Apparently under these conditions hydrocortisone caused atrophy of striated musculature by a direct topical effect.

Pincus: Is this the acetate or the free alcohol?

Selye: The acetate. The surprising thing was that although the skin became extremely thin it never perforated. Apparently despite its thinness it was highly resistant.

It seemed to us that this technique might be quite interesting for the bioassay of extremely small amounts of antiphlogistic or glucocorticoids. If the skin is merely insufflated and microcrystals deposited, a very small amount of hormone can be tested for its ability to cause atrophy of connective tissue even in the absence of any inflammatory phenomenon. For bioassay purposes we take a sheet of cardboard and after excising the skin detached by the pneumoderma technique we fix it to the cardboard with a stapler in a flat position. Then the cardboard with the skin is immersed in formalin or some other fixative. After fixation the flat sheet of fixed skin, which having lost its elasticity maintains its shape, is punctured several times with a paper puncher in the area immediately surrounding the hydrocortisone deposit as well as in the immediately surrounding but still detached area which was not influenced by the hormone. The small disks which have been thus punched out can be weighed exactly on an analytical balance. The mean

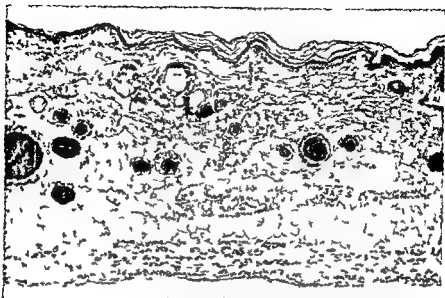


FIGURE 8. Microscopic appearance of the skin detached by the pneumoderma technique. This section has been taken at a distance from the hydrocortisone deposit.

the actions of hydrocortisone so applied were essentially similar to those described by Drs. Baker and Ingle after direct application of adrenocortical extract or of purified glucocorticoids to the skin in alcoholic solution. However, such topically administered corticoid solutions like the subcutaneous injections of hydrocortisone under normal skin exert their effects only at the site of application without the peculiar phenomenon of the spreading atrophy seen in these pictures.

Figure 8 shows the essentially normal structure of the detached skin in the pneumoderma region when taken from a region far away from the hormone deposit. There was some atrophy of the subcutaneous fat tissue and the hair follicles were perhaps not quite as numerous and well developed as they would be normally. Otherwise the skin retained its usual appearance. The slight inflammatory changes (some round cell infiltration particularly underneath the muscular layer of the skin) were probably due to repeated palpation of this region. They were not noted in many other experiments in which special care was taken not to injure the derma mechanically.

Figure 9 shows a similar skin section taken from the same animal but from the atrophic skin area immediately surrounding the hydrocortisone deposit. Here there were no hair follicles or sebaceous glands. Inflammatory changes were completely suppressed although it was

posit This in itself shows that the amount absorbed and responsible for the extreme atrophy in the surrounding skin area must have been very little

Rall Dr Selye is this a drawing of the microphotograph and what staining has been used?

Selye That is the microphotograph itself Hematoxylin eosin stain was used It looks like a drawing because the photograph was taken with a very narrow diaphragm which brings out the contours sharply

Sayers Wouldn't it be possible to extract the tissue and analyze for 17 hydroxycorticosterone?

Selye It would

Sayers Have you done studies of that type?

Selye No we have not

I should like to show a modification of this technique which we call the granuloma pouch If a smaller amount of air is injected let us say 25 ml into the loose subcutaneous tissue of the interscapular region a circumscribed air bubble is formed This can be transformed into a veritable granuloma pouch by the subsequent injection into the lumen of some irritant such as kaolin formalin or croton oil Usually we inject 1 ml of peanut oil or corn oil containing 1 per cent croton oil This causes pronounced irritation and transforms the loose connective tissue which surrounds the air bubble into a thick, inflamed membrane This membrane becomes well delimited from the surrounding tissue and the air in its cavity is gradually replaced within 14 days by a fluid exudate

We feel that the granuloma pouch technique has an advantage over the usual procedures for the study of experimental inflammation in that it permits us to produce consistently an accurately measurable granuloma mass and exudate Furthermore it facilitates the objective and accurate estimation of absorption from the inflamed surface into the blood or lymph stream as well as the estimation of the excretion of blood borne products through the inflamed barrier We have used the procedure particularly for the objective assessment of the functional value of granuloma tissue for instance its resistance to the topical effects of microbes chemical and mechanical irritants etc

In connection with the antiphlogistic effects of corticoids it is especially noteworthy that if hydrocortisone is injected subcutaneously as in the previously mentioned pneumodermal experiment and the loose connective tissue containing the hydrocortisone deposit is detached from the underlying tissues with 25 ml of air the subsequent injection of an irritant into this bubble no longer produces any definite inflammatory changes

difference between the weights of the disks coming from the thin area and from the surrounding skin respectively is taken as an objective indicator of the degree of cutaneous atrophy thus produced. To give an example the average weight of such disks in this experiment was 32.4 mg (with a standard error of 2.1) in the detached skin, just outside of the region directly influenced by hydrocortisone, and 11.1 mg (with a standard error of 0.9) in the atrophic areola surrounding the hormone. I might mention incidentally that if some inflammatory infiltration occurs in the detached skin as a result of handling it is very often predominantly eosinophilic.

Sayers: This is without hydrocortisone?

Selye: Without hydrocortisone. No, actually I should say it is with hydrocortisone but in a region of the skin which is at a great distance from the hydrocortisone deposit.

White: Have you done this serially so that you know eosinophils are the cells which predominantly come in all along after the injection of the area?

Selye: No.

White: These illustrations are from animals studied how long after the injection of the area?

Selye: This particular one (Figure 9) is at two months. I haven't followed the response longer.

I mentioned these experiments in connection with the mechanism of action of corticoids because it has always impressed me as a definite gap in our investigations that no one has as yet adequately studied the influence of hormone fixation in tissues. It should be emphasized that quite apart from dystrophic changes in the pneumoderma absorption from it is greatly delayed. For instance a hydrocortisone deposit which persists several months in the connective tissue of the pneumoderma would not persist for more than a few weeks if it were injected into normal subcutaneous tissue without air insufflation. Obviously the procedure fixes the hormone deposit at the site of the injection perhaps as a result of a locally impaired blood supply. The question arises whether differential fixation of hormones can occur naturally in various tissues of the same organism. If so selective effects upon individual targets would be explained even if the blood level or urinary excretion of the hormone remained unchanged.

It is obvious that if such fixation is artificially induced by the pneumoderma technique the hormone becomes extremely effective. However we have no proof that this is exclusively due to fixation. Presumably the nutritional disturbances in the skin also act as conditioning factors. At any rate in the whole course of this prolonged experiment we were unable to detect any visible diminution in the size of the de-



FIGURE 11 Macroscopic appearance of a fresh granuloma pouch in an untreated rat (left) and one in which inflammation was inhibited due to the injection of hydrocortisone into the granuloma wall (right)

dissect it as a complete spheroid membrane. I inserted a tape measure into the lumen of the membrane I did manage to dissect to show that it is so thin that one can easily see through it. Actually this is almost completely unaltered ordinary connective tissue lined by epithelioid cells.

To me the surprising thing is that in ordinary skin the topical effect of hydrocortisone is limited to the immediately adjacent area. In the dystrophic skin of the pneumoderma this effect spreads considerably further, and in the granuloma pouch the spreading is even greater. The hormone was deposited in a very limited area at one point of the pouch and it influenced all the connective tissue surrounding the air bubble even that situated several inches away at the pole opposite to the place where the hormone was deposited. At the same time exudate formation was virtually absent. Even after 14 days the cavity contained only air and the irritant which was originally injected.

Manifestly, under these particular circumstances the hydrocortisone managed to condition the influence of a very large surface of connective tissue. Yet its effect was still topical, since injection of the same amount of hormone into normal subcutaneous tissue at only a few millimeters from the pouch wall remained completely without effect, permitting the development of a thick, inflamed granuloma with a voluminous exudate.



FIGURE 10 Macroscopic appearance of a granuloma pouch after fixation and evacuation of the contents

As can be seen from Figure 10 the granuloma pouch produced with 25 ml of air plus 1 ml of 1% croton oil can easily be enucleated by simple pressure after splitting the skin surface. The pouch was fixed *in toto* and opened by a partial transverse section to show the regularity of both the outer and the inner surface.

Figure 11 shows on the left a similar granuloma pouch without cortisone treatment. Here can be seen the exudate level as distinct from the lighter area occupied by the residue of air because the tissue is not fixed—it is not seen distinctly because of the thickness of the pouch wall. On the right is the corresponding structure from a rat treated exactly the same way as that whose pouch is shown on the left but here one milligram of hydrocortisone was deposited into the loose connective tissue surrounding the air bubble. This structure—it can hardly be called a pouch—is extremely thin and therefore I could not

Pincus In the hydrocortisone area is there an inhibition of hair growth?

Selye Oh yes In the hydrocortisone areola there is never any trace of hair growth whether a pneumoderma is produced or a granuloma pouch This however only confirms what Drs Ingle and Baker have shown by topical painting of the skin with solutions of corticoids

Long Have you any idea what the air pressure is within that sac particularly in relation to the venous pressure?

Selye We have never actually measured it

Long Offhand one would think that the vein would collapse when the air pressure gets higher than the venous pressure

Selye When the air is injected there is very little pressure immediately afterwards The pouch is soft It is easily compressed between two fingers However if the pouch is irritated for instance by the injection of croton oil as shown in Figure 11 then the pressure becomes very high This is partly due to exudate formation and partly to the secondary contraction of the granuloma pouch which occurs after much fibrous tissue develops in its wall In those animals in which the pouch was irritated with croton oil but protected by hydrocortisone the outstanding histologic feature was the virtually complete absence of connective tissue fiber formation and hence of contraction The extremely thin wall in these cases consisted almost exclusively of roundish or polyhedral epithelioid cells Since this structure was incompatible with a cicatricial contracture the hydrocortisone protected pouches were always extremely flabby and of course devoid of exudate If the pouch was filled only with air and was not otherwise irritated then it remained quite flat and the pressure in it was never very high

Conn Are you going to try DCA and compound F mixed together and injected into the same area?

Selye I have done that I did not plan to report on it as yet because the DCA is absorbed too rapidly and I am not quite sure about the interpretation of my results The experiment was done with the ordinary pneumoderma not with the granuloma pouch technique and DCA and hydrocortisone were injected in the area simultaneously after being mixed in the same syringe The skin atrophy was just as marked as if no DCA had been injected

I forgot to mention that various irritants produce qualitatively different types of granuloma tissue Thus formalin tends to cause necrosis or at lower dose levels predominantly fibroblastic reactions croton oil a predominantly hemorrhagic exudate with an intensely fibrous capsule mustard powder results in the production of innumerable foreign body giant cells turpentine elicits predominantly pus formation We are now trying to examine the effects of various hormones on these

Pincus When formalin is used as the irritant is it injected into the air cavity the pneumoderma?

Selye Yes it is injected into the cavity immediately after the air is and then the animal is shaken up in order to distribute the irritant equally on the surface of the cavity

Sayers The irritant does not fill the cavity?

Selye No

Li Have you injected growth hormone into the pouch?

Selye I have but it is very rapidly absorbed and nothing happens Perhaps one should administer it simultaneously with substances which delay absorption If growth hormone is repeatedly injected outside the pouch, just subcutaneously in the same animal there is a marked skin hypertrophy which is not limited to the area of injection as Drs Ingle and Baker have shown

Pincus But if it is injected locally?

Selye Then a single injection is given which is very rapidly absorbed You see it isn't only that absorption is delayed in the detached skin but that hydrocortisone acetate is very slowly absorbed anyway If hydrocortisone acetate is put into the wall of the pouch the absorption rate is as slow as can be A water soluble substance like growth hormone is too readily absorbed for such single injection experiments If a growth hormone tannate could be made as there is a pitressin tannate or some other slowly absorbable preparation of STH then one might be able to use it As it is it cannot be employed for this kind of work

Thorn Is the circulation in the pouch too slow to permit the gas content of the pouch to be used for respiratory studies?

Selye Comparing arteriovenous values?

Thorn I was thinking of over all respiratory quotient studies

Selye We have made no chemical measurements on the pouch air If one compensates for the absorption of the air by injecting additional quantities a pouch can be maintained for months

Thorn I was thinking that you might be able to use the air in the pouch for the purpose of making respiratory quotient studies following the feeding of food and so forth which would bypass the technical difficulties of mass respiratory quotient studies in animals

Selye I think the oxygen is absorbed from the pouch very rapidly while the nitrogen stays behind In any case we have not done any study of this kind but I may say that although the detached part of the skin is poorly vascularized the base of the pouch is very rich in vessels

Vogt What happens to the hair Dr Selye?

Selye We usually pluck the hair or remove it with barium sulfide But it can grow back even in the area of the pneumoderma although it is greatly delayed there

Chow) *ad lib* After 15 days when this experiment was terminated their body weight was 136 Gm and their adrenal weight 35 (± 17) milligrams Group II like all the other groups was pair fed with group III that is they received just as much food as was required to maintain their body weight approximately equal to that of group III Since group III received cortisone and grew very little this meant a considerable artificial reduction in the food intake of group II At the end of the experiment the mean body weight in this group was 112 Gm and the adrenal weight 27 (± 12) milligrams

Group III was treated daily subcutaneously with 25 mg of cortisone acetate Here the final body weight was also 112 Gm The adrenals underwent the usual compensatory atrophy weighing 16 (± 11) milligrams These rats received food *ad lib* but grew very slowly because of the cortisone overdosage

Group IV received the same amount of cortisone as group III but they also were simultaneously treated with 4 mg of Armour's STH daily subcutaneously Their food intake was likewise so reduced by pair feeding that their growth curve was essentially similar to that of the rats in group III Their body weight was 116 Gm and their adrenals weighed 26 (± 16) mg at the end of the experiment

Group V received STH alone at the same dose level as group IV Here again the food intake was restricted so that their body weight remained essentially at the same level as that of the rats in groups III and IV At the end of the experiment their mean body weight was 112 Gm and their adrenals weighed 41 (± 21) milligrams

These figures I think conclusively prove that the corticotropic effect of STH is not merely a secondary consequence of its growth promoting action I use the term corticotropic not in the sense of a direct effect upon the adrenal cortex although this is not excluded but merely to denote adrenocortical stimulation whether mediated through the pituitary or not In any event the adrenal cortex enlarged under the influence of STH even if the growth promoting effect of this hormone was artificially prevented by reducing the food intake Under the same conditions that is by equalizing the body weight by restricted feeding STH also antagonized the compensatory adrenocortical atrophy normally induced by cortisone Let me emphasize that this particular STH preparation had virtually no corticotropic effect in hypophysectomized animals Hence I think it can be said as a general conclusion that its effect was not due to contamination with ACTH and that whatever the mechanism through which STH enlarged the adrenal of the intact animal it was not merely because it acted as a growth hormone

Long There was no growth promotion difference with STH between II and V?

different types of response to topical irritation using the granuloma pouch technique

Pouches can also be produced by microbes or you can purposely infect a pouch which is or is not under the influence of hormone therapy study and thus the functional effect of hormones on the spread of an infection. I need not go into all the obvious endocrinologic implications of this. We have also tested the effect of systemic treatment, i.e., injections at a distance from the pouch of cortisone, ACTH and STH. In these experiments the hormones were injected daily during the entire development of the pouch. As expected, cortisone and ACTH make the granuloma thin while STH causes it to proliferate and results in very thick walled granuloma pouches. We are in the process of using the same technique for the study of various other problems, for instance the effect of systemic stress upon granuloma and exudate formation.

Ralls: What size needle is used to form or inject the pouch?

Selye: We use a No. 25 hypodermic injection needle for the first insufflation of the pouch and the introduction of the irritant. However, after the granuloma is well developed it can be punctured with a heavier needle to remove specimens of the sometimes rather thick exudate which may not go through the No. 25.

I should like to present a few data on the interactions between cortisone and STH. In earlier studies we had noticed that if normally lethal doses of cortisone were given at the same time that STH was injected in quantities adequate to maintain a normal body weight then the lethal effects of the glucocorticoid were abolished or at least mortality was diminished. It will be recalled that Dr. Evans and Dr. Li showed some time ago that the effect of ACTH on the junction cartilages is opposed by STH. We found that the opposing effects of these two types of hormones upon inflammation, the spreading of infections and many other phenomena were also mutually inhibited in the case of simultaneous treatment with both types.

It is interesting that even the adrenocortical atrophy normally produced by cortisone can be abolished by simultaneous treatment with STH. This was rather unexpected and we wondered whether here STH acts upon the adrenals only secondarily as a consequence of metabolic changes connected with its growth promoting effect. We therefore attempted to eliminate the growth effect of STH to see whether it would still stimulate the adrenal cortex. In a very recent experiment we found the following. There were five groups each of eight female rats in this experiment and these were so selected that in each group the average body weight was 102 Gm at the beginning. Group I consisted of untreated normal animals which received food (Purina Fox

STH become unusually large even if the animals receive food *ad lib* and grow at an excessive rate. As a matter of fact it is under these conditions that we first noticed this corticotropic effect of STH.

Li In proportion to body weight though.

Selye Oh no, with high doses of STH the adrenals grow far more than in proportion to body weight.

Ingle Dr Selye, have you studied the effect of growth hormone on adrenal size in the hypophysectomized rat given a uniform intake of corticotrophin in amounts just sufficient to prevent adrenal cortical atrophy?

Selye No, but that is an experiment which Dr Salgado is doing now. I think we shall get definite results on this in a few weeks. One possibility is that the corticotropic effect of STH would manifest itself only if the adrenal was maintained in a responsive condition by STH treatment.

Ingle Was this Armour's growth hormone?

Selye Yes.

Sayers Dr Selye, could you not explain these data by a contamination of your STH with ACTH?

Selye No, because the same STH preparation when given in the same amount to hypophysectomized animals never gave any corticotropic effect in our hands.

Sayers You mean it is completely free from ACTH?

Selye Armour's tells us it contains a trace, but we could not detect it by using merely adrenal weight as an indicator. In any event the ACTH contamination in our STH must be so small that it could not account for the pronounced adrenal enlargement which I repeat we obtained only in intact animals. Maybe Dr Li knows how much it contains. I have forgotten the exact figure in units, but it contains very, very little.

Li A very, very small amount.

Selye It contains also a thyrotropic hormone.

Sayers I should like to know just how much it contains in order to evaluate an experiment of this kind.

Lukens I think that the figure for Armour's growth hormone is about one tenth of one per cent of ACTH on a milligram basis.

Pincus How much STH was given per day?

Selye That was 4 milligrams.

Sayers In two doses?

Selye In two doses.

Li The content of STH is very small.

Selye We went up to 5 mg. of the same preparation in hypophysectomized animals and obtained virtually no adrenal hypertrophy.

Selye They were purposely so fed as to have the same weight i.e. group V received much less food so that they would have the same weight as the animals in groups II, III and IV. That is what I meant by paired feeding in this case. The normal untreated rats in group I and the merely cortisone treated animals of group III were fed *ad lib*. All the others were fed in such a manner that more food was given when they became lighter than and less food when they became heavier than the merely cortisone treated animals of group III.

Thorn That is not pair feeding though.

Selye The food was arranged so as to arrive at the same final body weight. Pair feeding is perhaps not the right term.

Pincus Restricted feeding.

Selye How could we express the fact that their food intake was not merely restricted in an arbitrary manner but that they were fed so as to end up with the same body weight?

Pincus Controlled feeding.

Selye It should be corrected then and I should have spoken of controlled feeding.

Rull Do you happen to have the thymus weights on these animals?

Selye I do not have the figures with me because this experiment was terminated only a few days ago. We fixed the thymus glands for weighing as were the adrenals but up to now we have weighed the adrenals only.

Pincus Why do you conclude that this is a growth effect and not a nonspecific stress effect of the STH preparation?

L1 The food intake of the group V animals must have been tremendously smaller than that of group II so much smaller that they ended with starvation actually. What was the actual food intake each day in group V?

Selye We didn't measure it in this experiment but we are going to measure it in a similar one which we started after completing this and we shall publish our results in detail later. I may say however that the growth hormone treated animals required less food to maintain the same body weight. Would you have expected this?

L1 A growth hormone injected animal would eat more. So in order to restrict the diet for this animal to grow to the same extent as the untreated animal it would require much less food.

Selye It was our impression that they required much less food to stay at the same weight. Have you done any such experiments?

L1 Yes we have had some experience with that.

Selye It is of course possible as Dr Pincus said a minute ago that giving STH on a restricted diet which does not permit growth creates a situation of stress. However the adrenals of rats overdosed with

the body weight is relatively well maintained under these circumstances

Li It is still a stress because the animal requires more food in order to grow

Thorn If the caloric intake of a patient with myxedema is reduced this does not call forth the same reaction in the body as when the caloric intake is restricted to the same level in an individual whose metabolic rate is normal

Selye Pomeranz showed that the greatest adrenal hypertrophy occurs on complete acute starvation while chronic maintenance on a reduced food intake causes adrenal atrophy. If food is completely withheld there is marked protein catabolism and the adrenal becomes very large. This is presumably a manifestation of stress. In other words it might be stated this way: growth hormone causes an adrenocortical hypertrophy whether growth is permitted by giving food *ad lib* or restricted by maintaining the animal on an insufficient food intake. If you want to call this reaction a stress effect I agree but then we would have to say that whatever metabolic changes growth hormone effects whether causing growth or not it always is a stressor for the body and it always causes adrenal growth. By this time I think the whole matter becomes a question of semantics.

Pincus It is not a question of semantics if you assume that this stress involves ACTH release. You are not talking about definitions you are talking about the production of something that stimulates the adrenal.

Selye I think STH does cause ACTH release since in the hypophysectomized animal it does not produce adrenal hypertrophy and in the intact animal no matter what happens to growth it does cause adrenal hypertrophy. I think the most plausible explanation is that STH has as one of its specific effects the ability to induce a considerable ACTH discharge from the hypophysis. However the manifestations of this ACTH discharge are largely blocked because of the antagonism between STH and ACTH as regards their effect upon most organs. In our very early experiments with LAP we were struck by the production of a singular set of symptoms namely very much enlarged adrenals generalized inflammatory changes myocarditis arteritis arthritis a large thymus and a large spleen. This combination of changes could not be ascribed either to ACTH or to STH. It was difficult to explain and we could only say that it was the result of treatment with an impure pituitary preparation. Now however we assume that it was due largely to STH which is plentiful in LAP and to the endogenous ACTH released under the influence of the former.

Pincus We have perfused adrenals with LAP and found no measurable effect on steroid release. This agrees with the direct effect of LAP of which you speak.

Sayers If one unit is taken as equivalent to one milligram of the standard preparation the rats received 1 milli units of ACTH per day. This is not an insignificant quantity of ACTH.

Astwood That would have no effect on adrenal weight would it?

Selye Especially if given in only two injections.

Pincus It would have a marked effect on the ascorbic acid depletion.

Sayers Five milli units injected once per day have an appreciable effect on adrenal weight in the hypophysectomized rat (10).

Selye Fourteen milligrams of adrenal weight were added between groups II and V although the final body weight was the same. If a larger dose namely 5 mg. of the same STH is given to hypophysectomized rats under the same circumstances by two injections a day in water as we have done it here there is no appreciable increase in adrenal weight. I do not doubt that it might deplete ascorbic acid.

Sayers The administration of cortisone reduces adrenal weight and in a sense produces the same situation as in a hypophysectomized rat. We know that the hypophysectomized animal is much more sensitive than an intact animal to small doses of ACTH as far as adrenal weight is concerned. The administration of cortisone is a sort of chemical hypophysectomy.

Selye If you consider it as such then the difference between group III and IV should be duplicated on similar animals after hypophysectomy and that we didn't find to be the case.

Pincus May I ask for an answer to the question I proposed originally. Why don't you interpret this as a nonspecific hypertrophy of the adrenal rather than as a specific growth hormone effect in group V?

Sayers The effect is probably the result of endogenous release of ACTH.

Pincus Yes.

Selye In the animal which is fasted it may be argued that the animal is under greater stress if growth hormone is simultaneously given than if it is fasted without the growth hormone. This case could be interpreted that way. But I am not very much inclined to ascribe the whole STH effect to stress under all conditions because as I said before even if free growth is permitted and the animal is obviously in fine condition there is still the corticotropic effect.

White The question could be answered if there were one more group of animals in group I restricted to the same food intake as is group V.

Thorn If growth hormone prevents weight loss despite a reduction in food intake it really is not an added stress is it? In other words these animals are not starved more than control animals in the group. The stress of not eating must be considerably less on the organism if

the body weight is relatively well maintained under these circumstances

Li It is still a stress because the animal requires more food in order to grow

Thorn If the caloric intake of a patient with myxedema is reduced this does not call forth the same reaction in the body as when the caloric intake is restricted to the same level in an individual whose metabolic rate is normal

Selye Pomeranz showed that the greatest adrenal hypertrophy occurs on complete acute starvation while chronic maintenance on a reduced food intake causes adrenal atrophy. If food is completely withheld there is marked protein catabolism and the adrenal becomes very large. This is presumably a manifestation of stress. In other words it might be stated this way: growth hormone causes an adrenocortical hypertrophy whether growth is permitted by giving food *ad lib* or restricted by maintaining the animal on an insufficient food intake. If you want to call this reaction a stress effect I agree but then we would have to say that whatever metabolic changes growth hormone effects whether causing growth or not it always is a stressor for the body and it always causes adrenal growth. By this time I think the whole matter becomes a question of semantics.

Pincus It is not a question of semantics if you assume that this stress involves ACTH release. You are not talking about definitions; you are talking about the production of something that stimulates the adrenal.

Selye I think STH does cause ACTH release since in the hypophysectomized animal it does not produce adrenal hypertrophy and in the intact animal no matter what happens to growth it does cause adrenal hypertrophy. I think the most plausible explanation is that STH has as one of its specific effects the ability to induce a considerable ACTH discharge from the hypophysis. However the manifestations of this ACTH discharge are largely blocked because of the antagonism between STH and ACTH as regards their effect upon most organs. In our very early experiments with LAP we were struck by the production of a singular set of symptoms: namely very much enlarged adrenals, generalized inflammatory changes, myocarditis, arteritis, arthritis, a large thymus and a large spleen. This combination of changes could not be ascribed either to ACTH or to STH. It was difficult to explain and we could only say that it was the result of treatment with an impure pituitary preparation. Now however we assume that it was due largely to STH which is plentiful in LAP and to the endogenous ACTH released under the influence of the former.

Pincus We have perfused adrenals with LAP and found no measurable effect on steroid release. This agrees with the direct effect of LAP of which you speak.

I should like to ask what histologic effect there is on the group V adrenals when they are compared with the others? If an ACTH effect is present, there might be indications from such things as lipid depletion

Selye They are rich in lipid

L How about the medulla?

Selye We have never seen any change in the medulla. That is another thing which is worth mentioning. The figures which you have published (11) are perfectly convincing to me but we never obtained such changes

L If the hormone is injected for a period of over a year say four teen months in rats the weight of the adrenals is about double their original weight. But as for the histology only the medulla is hypertrophied not the cortex

Lukens Dr Selye would it clarify this situation and answer Dr Styers question if these experiments were conducted with 1 mg of growth hormone a day? This is known to be an amply effective dose for growth and nitrogen retention in the rat. This dose would contain so little ACTH if our experiments are correct that it would answer the objection of contamination with ACTH. Has that been done? Have you tested 1 or 5 mg of growth hormone for its ACTH content in hypophysectomized rats?

Selye We gave up to 2.5 mg twice daily in aqueous solution to hypophysectomized animals without obtaining any significant increase in adrenal weight. We did not determine the ascorbic acid of these adrenals. However under exactly similar conditions in the intact animals the adrenals become very large as a result of the same STH treatment. If ACTH is given without any retarding agent in aqueous solution twice daily it is singularly ineffective

Long I was very much interested in Dr Selye's statement as to the inhibition of the effects of cortisone by growth hormone but I believe I know one situation where they apparently do not inhibit but rather synergize each other and that is in the production of glycosuria. It has been shown I think by three different laboratories that in the rat the dog and the cat where either hormone alone is relatively ineffective a combination of the two will produce quite a marked glycosuria. Perhaps in the same vein one might say that as Dr Selye mentioned in the absence of the hypophysis these DCA effects are greatly reduced. Certainly the effects of cortisone on carbohydrate and protein metabolism appear to be just as marked in the hypophysectomized animal as they are in the normal animal

Selye We have done blood sugars on animals chronically treated with cortisone and STH and as far as I can remember we have never

had a blood sugar over 180 under our experimental conditions in the rat. Yet in general the blood sugar of animals simultaneously treated with STH and cortisone tended to be higher than that of controls receiving cortisone alone. Of course I am far from believing that all the effects of STH are antagonized by cortisone and vice versa. I am well aware of the reported synergism as regards carbohydrate metabolism but there may be species differences and the rat is particularly resistant to the production of diabetes by this combination of hormones. Even differences in the strain of rats make for great variations as regard diabetes in the rat.

White Does someone recall better than I Dr. de Bodo's report at the Laurentian Hormone Conference? I had the impression that he said the diabetogenic effect of growth hormone in the hypophysectomized dog was counteracted by cortisone.

Selye Yes, that is correct.

Lukens That is correct except that I think he was measuring the action of insulin rather than the production of glycosuria.

L There are other situations where these two hormones act synergistically. One such situation is the appearance of ketonemia and ketonuria in fasted normal rats. As we have reported (12) both growth hormone and ACTH have ketogenic activity. Even in the absence of the adrenal the ketogenic effect of growth hormone is apparent. Another example is the increase of liver fat content in hypophysectomized and normal rats through the effects of these two hormones (13). I should like to comment on this antagonistic effect between cortisone and growth hormone. For a number of years we have been injecting growth hormone into both normal and hypophysectomized rats for periods of over a year's duration. We have found that in the normal animal tumors are produced with growth hormone whereas none are formed in hypophysectomized animals. We have been thinking recently that perhaps the adrenal or the gonads might be mediating factors in this phenomenon of the formation of tumor after long term injection of growth hormone.

Selye These are lymphatic tumors?

L Yes, but not only lymphatic. There have also been tumors present in the medulla, for instance.

For the past year or more Dr. W. O. Reinhardt and I have been injecting growth hormone into a group of castrated adrenalectomized rats whose pituitaries were still intact. After the first three months of injection we noticed that the animals had developed a condition with all the symptoms of chronic arthritis: the joints were swollen and their locomotion was greatly impaired. Radioautographic studies of the bone showed a typical arthritic condition. These symptoms were present in all of the adrenalectomized castrated animals which had been treated

with growth hormone in only one of the normal growth hormone treated controls and in none of the untreated controls. We took two of the adrenalectomized castrated animals which were in poor physical condition and injected them with 1 mg of hydrocortisone. Within two or three days this treatment seemed to relieve the symptoms. The growth rate of these animals continued to be the same as that of normal animals with intact adrenals. This experiment seems to show that growth hormone does participate to a certain extent in arthritic conditions in the rat and that hydrocortisone in some way counteracts this effect of growth hormone.

Selig: What type of arthritis is it?

L: I cannot say since the animals are still being injected with growth hormone and the tissues have not yet been studied. The arthritic results were only incidental, the primary object of the experiment being the production of tumor in adrenalectomized-castrated animals. However it is interesting to note that in not a single one of the normal animals treated with growth hormone has there been an arthritic condition produced; this condition occurred only in the absence of the adrenals or when they were atrophied as a result of hypophysectomy. It would appear that the growth hormone had no alarm effect on the animal.

Corn: How were these animals maintained?

L: With saline.

Rall: Did you say you had to castrate as well as hypophysectomize the animals in order to produce the arthritis?

L: We don't know. These are, as I said, incidental observations. The experiments were performed because we had thought that tumor formation might be due to the production of a gonadotropic substance or to the production of a certain form of ACTH by the pituitary.

W. Bate: The reason I asked previously about the ketogenic effect is that I rather like the idea of a balance between growth hormone and ACTH in the sense that a balance of factors fits in with the general picture of some part of a homeostatic mechanism. I recall that we ourselves and other people have described for example a marked effect of cortisone or adrenocortical extract on lipid mobilization. One begins to wonder how many of those experiments must be repeated in the hypophysectomized animal in order to determine whether those data are attributable to a secondary response of endogenous growth hormone in an attempt to counteract the effect of cortisone.

The capacity of the human pituitary to produce ACTH might account in part for the fact that growth hormone has to the present time produced very little effect on man. Could it be that there is a degree of ACTH production in response to growth hormone injection in man which results to some degree in a negation in this species of any effect

that might be expected of growth hormone?

Ralli It might be a matter of the dose

W. Hite Yes in any balance of this type there is generally a quantitative relationship. I don't know that anyone would want to try or has tried using heroic doses of growth hormone in man. I don't know if it would be particularly advisable. But one does wonder whether there is a dose level at which man may respond to growth hormone. I think that the first doses of cortisone which were used by the Mayo group in rheumatoid arthritis were considered at the time to be rather heroic doses and perhaps the same situation obtains with respect to growth hormone.

Ralli A situation that gives a clue to dosage may be the overgrowth of tissue that occurs in the patient with acromegaly which is due to an eosinophilic tumor of the pituitary. I do not know of any recent assays for the growth hormone activity of these tumors nor do I know what other pituitary hormones might be increased in this situation. It would appear that the growth hormone is decreasingly effective as maturity is approached but in the acromegalic who is a mature individual an overgrowth of bone and of other organs is produced i.e. the liver the kidneys the spleen.

Selye In connection with Dr White's remark concerning the insensitivity of the human being to STH it is perhaps of interest that based on a very similar theoretical consideration we have given STH plus DCA on the assumption that DCA does not have any catabolic effect but does cause compensatory adrenal atrophy and might to some extent compensate for any ACTH secretion that the STH would induce. We found in fact that small doses of DCA given to nonsensitized animals increase the growth effect of threshold doses of STH in the rat.

A second relevant fact is that in recent experiments performed in collaboration with Drs Mitchell and Guillemain we found that the guinea pig is entirely insensitive to the growth effect of such STH preparations as are now available. This is apparently true even of hypophysectomized animals. It might be interesting to use the guinea pig as a test object to work out those conditions under which a growth hormone resistant species might be made sensitive to this principle. Let me add that the same STH preparation which was ineffective in the guinea pig proved highly growth promoting in the rat under exactly comparable laboratory conditions.

Conn Commenting on Dr White's hypothesis—it is true that by and large administration of growth hormone in man has not resulted in positive nitrogen balance even though the same preparations were growth promoting in animals. In fact humans have frequently demon-

with growth hormone, in only one of the normal growth hormone treated controls and in none of the untreated controls. We took two of the adrenalectomized-castrated animals which were in poor physical condition and injected them with 1 mg of hydrocortisone. Within two or three days this treatment seemed to relieve the symptoms. The growth rate of these animals continued to be the same as that of normal animals with intact adrenals. This experiment seems to show that growth hormone does participate to a certain extent in arthritic conditions in the rat and that hydrocortisone in some way counteracts this effect of growth hormone.

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of the normally mobile rat skin due to this fibrous tissue proliferation

Astwood Have you not had the impression though that one preparation may differ from another in the extent to which it produces this binding down of the skin although the preparation might exhibit comparable growth hormone activities?

Selye I could not answer that because we have used only Armour's preparation for experiments in which we specifically injected at the same spot daily. Unless this is done such an effect is not obtained anyway.

Ralli Are there any data on the secretion of growth hormone in varying age groups? If the elderly rat were to be studied would a growth hormone assay of the pituitary indicate that it had decreased in amount? Is there any way of assaying throughout the life span the rate of secretion of the growth hormone?

L Dr Ralli we once spent a whole year trying to detect growth hormone activity in various subjects ranging from children to people in old age without success.

Long In the blood?

L Yes these were blood studies. However Dr Kinsell and I (14) made a study of patients with active acromegaly where we did find some growth hormone. We still couldn't detect any in the urine but we found a high growth hormone content in the plasma.

Long Is it possible to get a figure for the content of growth hormone in the rat pituitary? Is there enough there? I think that what Dr Ralli was getting at was whether there was any change with sex or age and so on.

L We haven't really tested that. We have done only a few selected experiments so far. That is one reason why we have tried to improve our assay method by sensitizing the animal with thyroxine.

Long P. A. Smith placed half a lobe of pituitary under the skin of a hypophysectomized rat. The next day he observed quite a sharp increase in weight.

L Growth hormone is an extremely labile protein. One of the criteria of denaturation is the change of mobility in electrophoresis. This phenomenon can be demonstrated by a simple experiment. If a growth hormone preparation in a pH 9.5 buffer is maintained at room temperature for two days in a sterile condition there will be no change of activity but a new component having a higher electrophoretic mobility will appear.

Long Would that prevent destruction of a pure form? After all tests for the principles of growth hormone are fairly sensitive.

L I don't know why growth hormone activity cannot be detected in a single rat pituitary although we can detect 5 gamma activity of

strated mild negative nitrogen balance when given growth hormone. This latter finding forced us to consider the same point raised by Dr White even though administration of growth hormone produced no eosinopenia or other measurable increase in adrenocortical activity. We therefore gave growth hormone to a patient with Addison's disease and failed there also to induce a positive nitrogen balance.

Selye I may say that the lack of growth promoting effect observed in the guinea pig was probably not due to thyrotropic contamination in our STH. The extract we used for this particular work was made by Armour specifically with the view of testing a preparation as free of thyrotropin as possible. Although I cannot give exact figures, it is extremely low in thyrotropic activity.

Long Dr Astwood knows as much about these new preparations of growth hormone and their lack of effect in man as does anybody. It is my understanding that they did produce nitrogen retention in man.

Astwood They have sometimes induced a positive nitrogen balance but not always. It seems to me that growth hormone preparations vary widely in what might be called their toxicity. Some of them at least in man are toxic in the usual sense of the word producing either local inflammatory reactions or fever. If a preparation can produce fever in one patient it seems to me quite possible that it might be producing some noxious influence in another even though there may be no actual elevation in body temperature. Increase in sedimentation rate for example would indicate an effect that is probably related to toxic impurities. Raben and Westermeyer* have evidence that current growth hormone preparations can be fractionated to give apparently different chemical fractions. Their work on human beings has been deferred pending further purification. I should like to ask Dr Selye whether he has studied the sites of injection of large doses of growth hormone in animals whether there is not a local inflammatory reaction which varies in its extent from one preparation to the next?

Selye Our attention was called to the site of injection in those experiments in which we gave stilbestrol and STH at the same time. Fibroma like local connective tissue proliferation developed at the site of STH injection. Repeated injection of STH into the joint region also caused marked fibrous tissue proliferation in rats. In all these experiments it was very difficult to distinguish between nonspecific local irritation, some allergic reactions and specific growth hormone effects. However histologically the tissue developing at the site of STH injection was almost entirely fibrous and virtually devoid of any true inflammatory component. Repeated injection of STH in the same subcutaneous region for instance on the back of the animal caused a binding down

*Raben, M. S. and Westermeyer, V. W. Unpublished data.

Thorn : Dr Robert Gaunt has made some interesting observations on the effects of various steroids in restoring the adrenal weight in the presence of cortisone inhibition*

Selye That is methylandrostenediol

Thorn That is one of the compounds. There are several which are quite effective

Selye It must be remembered that methylandrostenediol causes a change in the adrenal qualitatively different from that produced by ACTH. So I don't think it can be interpreted as an effect caused exclusively through a discharge of ordinary ACTH.

White : Regarding the existence of several kinds of ACTH (1) Sulman (15) states that evidence exists for three corticotrophins: an adrenal ascorbic acid depleting factor, an adrenal weight increasing factor and a chromatotropic factor. What is the opinion regarding this problem? I believe that Dr Li and Dr Ingle have done work suggesting that there is a factor which lowers the adrenal ascorbic acid and that there is a separate factor which maintains adrenal size. Dr Young's group at Cambridge have similar evidence. I think Dr Li also has indicated that there may be a separate eosinopenic factor. I wonder whether in these studies this is a dose response factor or whether the evidence is adequate that these are different substances. In other words, may the same substance produce a diversity of effects depending upon the dose level?

Li : I must say that I was somewhat surprised to read Sulman's paper, especially his remarks about the third factor. I think Dr Astwood will agree that the ascorbic acid depleting factor is almost free of a chromatotropic effect, or in other words, that the intermedin component is not the same as the ACTH component.

With regard to the adrenal weight factor and the ascorbic acid depleting factor, I don't think that there is any definitive evidence to the effect that they are separate chemical components, although it is suggested by evidence from chemical studies that they might be. In our own experiments we found, for example, that when a highly active ACTH preparation, let's say a preparation of a hundred units per milligram, is digested with acid, there is in some cases an enhancement of ascorbic acid activity but with an accompanying loss of adrenal weight maintaining activity. On the other hand, when ACTH is heated in dilute sodium hydroxide, the ascorbic acid activity of the fraction almost disappears while the activity of the adrenal weight factor and the eosinopenic effect appear to be quite resistant to the treatment. These observations are not sufficient to provide evidence for these factors.

*Personal communication

purified growth hormone by means of the tibia test. It was also very surprising that we couldn't detect any growth hormone activity in the blood of young children.

Thorn: What are the comparable quantitative requirements in your best preparations of growth hormone and ACTH as indicated by the response in the rat?

Li: We are just looking at that quantitation so to speak.

Thorn: Can you give an approximation?

Li: No, that is a very difficult problem because the effect of ACTH depends so much on the rate of absorption. Apparently, growth hormone is not very much affected by this factor, in fact our experience has been that even when growth hormone was injected three or four times a day the potency of the hormone was not changed to any great extent. But with ACTH it is a very different matter. I do not know what the best conditions would be to control this absorption factor.

Long: Dr. Selye outlined certain possibilities among them the possibility of a plurality of ACTHs. We touched briefly before on the question of there being an eosinopenic ACTH, an ascorbic acid lowering ACTH and an adrenal weight increasing ACTH. Would anyone like to comment further on whether there is one ACTH or whether we have to think of its effect as due to several compounds? How about it, Dr. Astwood?

Astwood: I don't know. We have been trying to purify what we have been calling corticotrophin, assuming that it is a single substance and so far we haven't encountered evidence that it is multiple. Dr. Selye's demonstration that giving a partially purified corticotrophic preparation together with a growth hormone preparation caused a completely different effect upon the adrenals again brings up the question, however, of a second pituitary factor acting upon the adrenals.

Selye: I should not like to give the impression that the effect is completely different. It is conceivable that the necrosis in the animals treated with both hormones was simply due to the fact that these adrenals were still more stimulated than those of the rats receiving ACTH alone and that some breakdown of the tissues resulted from overstimulation. In other words, I should not like to go on record as interpreting this experiment as definite proof of a plurality of ACTHs.

Thorn: Don't we need some reservations in this regard since we know of at least three substances which are capable of restoring the adrenal size to normal in the presence of pituitary inhibition: (a) exogenous ACTH, (b) growth hormone, (c) certain steroids particularly androgenic substances.

Selye: I have had no experience with androgens but as regards the other two I would certainly agree.

has asked about namely using different ACTH preparations. These were supplied by Dr. Astwood, Dr. Li, or were certain Armour preparations, and we have gone into a detailed analysis of the products by the method we described with Dr. Zaffaroni. Thus far we have, at comparable doses, seen no qualitative difference in the output of adrenal steroid no matter what ACTH preparation was used. On the basis of these experiments we tend to think that whatever differences may be exhibited by these adrenotropic hormones may in some involve this phenomenon, namely a tendency for production in one direction rather than another. We feel this rather strongly because in examining the enzyme systems which are responsible for the production of the various transformations ending in the active adrenal steroids we find that some should be clearly labile to one type of, shall we say, enzyme poison and others to an entirely different set. In other words, the 11β hydroxylase which we have been working on very actively has certain necessary co-factors for the operation of the enzyme, whereas the 21 oxygenating enzyme requires quite different co-factors. And one can conceive of a situation where a deficit of one co-factor might shift production from 11 oxygenation to 21 or even 17-oxygenation.

Ralli: Are these single experiments?

Pincus: This experiment has been done in about fourteen beef adrenals.

Bush: That is red triphenyltetrazolium and not the blue anisole type?

Pincus: The blue.

Ralli: In the work you reported several years ago, if I remember correctly, you gave ascorbic acid in the perfusing medium. When you perfuse with ACTH now, are you giving pure ACTH or are other factors included?

Pincus: We have been working with ascorbic acid added to the blood and we have seen no qualitative differences in the nature of the adrenal output. Mr. Fish and I have found that if ascorbic acid is added, the output can be sustained. In other words, with constant administration of ACTH, the adrenal tends to decrease its output, beginning at about the second to third hour of perfusion, but if ascorbic acid is added, the output can be maintained over a period of eight or more hours. That seems to be the only effect of ascorbic acid.

Ralli: Have you tried any other fractions that might be part of co-enzyme systems?

Pincus: Oh yes, we have, but I do not want to give a paper now.

Long: I am sure Dr. Ralli would like to know if you added pantothenic acid.

Pincus: Pantothenic acid has had no effect on the total output as far

being separate entities since they would have to be isolated in pure form before that could be stated for a certainty. But evidence so far suggests at least, that there might be different activities in different preparations of ACTH.

Vogt Is it conceivable to explain this type of result by assuming that the molecule has been modified so that its rate of absorption is changed? The ascorbic acid test is a very rapid one. A sudden rise in blood level of ACTH and then a rapid fall is wanted. On the other hand, for the adrenal weight increase, I think a constant rather low level of ACTH circulating from the site where it has been injected would be desirable. Is it just possible that this might explain the results?

Li That may explain the variation in ascorbic acid but in the case of the adrenal weight factor we always use a vehicle which allows for delayed absorption like a beeswax suspension in peanut oil when we inject animals to assay for the adrenal weight factor. I think then that it is quite clear in this case that the adrenal weight factor is lowered as the hormone is treated with an acid and maintained at the same level when it is treated with an alkali.

Long We are getting around to the point in which Dr. Bush and Dr. Pincus are very much interested. ACTH, one or three of them is producing the effects observed by increasing or changing the rate of secretion of adrenocorticosteroids. I wonder what evidence there has been on the effect of rate of secretion of adrenal steroids on these different types of ACTH preparations. Dr. Pincus perhaps we can begin with you and then Dr. Bush can tell us his experiences.

Pincus Some very recent work tends to substantiate that all ACTHs are the same but that different results can be obtained with the same ACTH. In regard to the latter point we have some preliminary data from Mr. Macchi of our laboratories that at a critical concentration (about 0.1 IU per liter) of ACTH in the perfusing medium a significant production of steroid glycol (or glycerol) may occur whereas at other concentrations (e.g. at 10 IU per liter) practically all of the steroid produced is a ketol. We arrive at this notion by comparing the titer of formaldehydogenic substance in the steroid extract with the titer of a ketol measured by the triphenyl tetrazolium reaction. The formaldehydogenic substance titer arises from formaldehyde generated from either ketol glycol or glycerol side chains at C₁₇ whereas the tetrazolium reagent appears to be specific for ketols. The formaldehydogenic substance minus the triphenyl tetrazolium reaction should therefore indicate the amount of glycol or glycerol. Differences in the nature of the adrenal secretory product may arise within the adrenal itself depending on the ACTH concentration supplied to it.

We have done the experiment a number of times which Dr. Long

TABLE I

Influence of Different ACTH Factors on Adrenocortical Secretion in Hypophysectomized Rats

Figures are total Δ^4 3 ketosteroid secretion rates in mg/Kg/24 hrs

	GROUPS				
	I	II	III	IV	V
A) Hypophysec tomized (1)*	0	0	—	—	—
1 day (2)*	0	6	—	—	—
B) Hypophysec tomized (1)	0	0	0	20	0
7 days (2)	0	0	0	20	0
C) Hypophysec tomized (1)	0	0	—	—	—
42 days (2)	0	0	—	—	—
	No treatment	AW factor	AA factor (aqueous)	AA factor (beeswax)	Placental ACTH

*1 and 2 refer to first and second samples of adrenal blood

weight while the placental factor had an effect very similar to the growth hormone which was to increase the adrenal weight or maintain it

Histologically these adrenals showed that those from the AW treated group were exactly the same as those obtained by Dr Renford. The gland was loaded with lipid and the lipid free zone inside the zona glomerulosa was abolished. In the AA treated ones there was little or no effect. With AA in beeswax there was lipid depletion and great dilation of the venous sinuses of the gland; the gland was hyperemic and lipid free in all the groups. The placental ACTH produced effects very similar to the growth hormone but there were slight histologic differences between the effects of the placental ACTH and the AW factor.

Finally the animals were anesthetized with ether and Nembutal; the left adrenal vein was cannulated and adrenal blood collected for two consecutive 20 minute periods. The second sample in each case was taken during stimulation with a continuous infusion over 20 minutes of an equivalent of 1 mg of Armour's ACTH.

In group I category A the first sample contained no detectable steroid. The second sample contained about 25 μ g of compound B and somewhere about 3 to 5 μ g of an unknown compound which is a Δ^4 3 ketone but does not reduce triphenyltetrazolium chloride and from certain other properties appears to be 20:21 glycol. The other untreated

as we could see at least in acute experiments. We have also used pantothenic acid the presumed precursor of co enzyme A and we have seen absolutely no effect from it. We at times obtain somewhat of an effect if we combine pantothenic acid with nor epinephrine but don't ask me why.

Long What kind of effect?

Pincus There is apparently an increased amount of steroid.

Thorn Where neither one alone produces it?

Pincus Yes.

Long Dr. Bush would you like to make some comments?

Bush Dr. Li (16), Dr. Selye (17), Prof. F. G. Young and M. Stack Dunne and Dr. G. Renford have all obtained ACTH preparations which tend to produce a great increase in adrenal weight and excessive deposition of lipids in the adrenal as distinct from the picture given by excessive doses of ACTH preparations which are active in the ascorbic acid depletion test. Professor Young and Stack Dunne have called their two preparations the adrenal weight factor and the ascorbic acid factor abbreviated as A W factor and A A factor. The difficulty is that there are two possible ways of interpreting these results. Dr. White has suggested one namely that the various end responses measured appear at different thresholds of dosage. The other interpretation brought up by Dr. Vogt is that it may be due to differences in absorption rate. Dr. Sayers (18) in a series of rather good diagrams showed that with different rates of absorption or different quantities and different time relationships with one single ACTH different histologic pictures and different effects on the weight of the adrenals can be expected.

Stack Dunne and I thought it would be good to try to check this by a method which would overcome the criticism of differences in absorption rate. The experiments were done as follows. Table I the animals were hypophysectomized 1, 7, 14 and 42 days before the final experiment. The groups consisted of about 5 animals each. One group was untreated throughout the whole period. They were all on a bread and milk diet which was found to keep these rats in very good condition. The second group was treated with the adrenal weight factor. The third group was treated with the ascorbic acid factor in aqueous solution. The fourth group was treated with the ascorbic acid factor in beeswax to retard the absorption. A placental ACTH preparation was given two times a day 1 mg subcutaneously to the fifth group. The same differences as Dr. Renford described although these were hypophysectomized rats were found with these different preparations.

In all these animals the adrenal weight was maintained or increased by the adrenal weight factor. The ascorbic acid factor either in aqueous solution or in the beeswax medium caused little or no change in adrenal

It appears that there are two ACTHs if they can be called adreno trophic but that one doesn't seem to troph or affect the adrenal in every way it might

Pincus I should like to make one remark about this disappearance of responsivity A phenomena which we have frequently noticed in the adrenal that is being perfused is the necessity for what we call a priming action If an adrenal is mounted and perfused immediately with ACTH with the first passage of ACTH through that adrenal there may be little or no increased steroid output depending apparently upon the condition of the adrenal If blood is circulated through that adrenal either with or without ACTH for one or two cycles and then the ACTH is given an increased output is observed Perhaps your second series might change to something positive if you had some means of priming

Bush You get similar cases clinically do you not?

Thorn Yes

Bush Dr Thorn has mentioned as an aside to me that the next thing is to take the same groupings of animals and do the experiment over again but treat the animals for longer periods It seems that although the weight factor maintained the adrenal it nonetheless did not have the priming action

Thorn Do you think that a mixture of A W and A A would give a value of 0.35 or 70 during the initial 20 minutes of observation?

Bush I think actually the the experiment to do is to treat the rats for a week with multiple injections of ascorbic acid factor in aqueous solution and stop the injections immediately before the experiment so that the secretion rate is low enough during the first sampling to obtain a subsequent response to another dose of A A factor What one wants to know is whether 24 hours after stopping treatment with A A factor alone the adrenal will again respond to A A factor

Rall Does the adrenal have to be conditioned before experiments are run?

Pincus We do it now routinely in order to get a maximum yield The yields I originally reported here ran about 2 to 3 mg formaldehyde genic substance per cycle The cycle lasted ordinarily for an hour Now our outputs run from 5 to as much as 10 milligrams In order to get up to that level we use the initial priming with blood We can never reach that high level immediately Sometimes indeed the output with ACTH is on the initial run through exactly the same as without ACTH

Rall What explanation have you for this lack of effect of ACTH? Could it be concerned with the enzyme systems within the cells which may need stimulation before they begin to operate?

Pincus I do not have factual data but I think essentially this means that the chain of enzyme systems which must operate in order to give

groups (IB and IC) showed no steroid in the first or second samples. In other words over a period of 20 minutes their adrenals not only secreted nothing initially but did not respond to the ascorbic acid factor.

White: Pardon me, Dr. Bush, I am not quite clear what you mean by samples.

Bush: Two samples were taken from each rat. In each case the A A factor was injected intravenously every 5 minutes during the collection of the second sample. In some animals a possible delayed action of the A W factor was tested by injection at the beginning of the first sample.

Pincus: Was this continuous treatment for 1, 7, 14, and 42 days?

Bush: Yes. The group numbers refer to the treatment given between hypophysectomy and the collection of adrenal blood up to the adrenal cannulation experiment.

Thorn: I am not clear as to the control group during the 7, 14, and 42 days. What happened to them?

Bush: In the untreated control groups no steroid was recovered from the first sample and no detectable steroid was recovered in groups IB and IC when ascorbic acid factor was injected. In other words there was an immediate and considerable response in this gland in the one day hypophysectomized group whereas in the others there was none.

The peculiar thing is that although the adrenal weight factor and the placental factor maintained the adrenal weight at the normal level or actually caused an increase and produced a gland which was rich in lipids and apparently in fine condition to start secreting whenever stimulated, the same behavior occurred in these A W treated animals as occurred in the untreated. The adrenal weight factor maintained the weight and histologic appearance of the gland but did not maintain the ability of the gland to respond to the ascorbic acid factor.

In the case of the ascorbic acid factor treated animals the ascorbic acid factor being given in aqueous solution, group 1A responded normally. But again there was no response in groups B and C which were hypophysectomized 7 and 42 days previously. The animals treated with ascorbic acid factor in beeswax showed little or no increase in adrenal weight and depletion of adrenal lipids. About 35 gamma was found in both the first sample and the second samples. The secretion rate in the first sample was so large that the injection of exogenous A A factor was not sufficient to produce any effect.

In none of these cases as Dr. Pincus also found was there any significant difference in the type of steroid released. It seems to indicate the rather curious result that there is a factor in pituitary extracts, the adrenal weight factor, which maintains an apparently normal gland or a gland well loaded with lipids and yet will not maintain the ability of that gland to respond to the factor which actually causes the increased secretion of these hormones.

be absorbed more slowly so as to give different effects but that even if injected intravenously it may depend on dissociation for its effect

Long One question I should like to ask goes back to work by Dr Astwood and Dr Tyslowitz (19) and also to some work by Dr Sayers showing that the ascorbic acid content of the adrenal one day after hypophysectomy is around 400 mg per cent and 42 days after as I recall it is in the neighborhood of 100 mg per cent. The same is true of the cholesterol content. Dr Sayers and Dr White remember that in the very early experiments we did with ACTH we found in one to three days a very good response in cholesterol whereas fourteen days afterwards there was a very poor response. Certainly the chemical composition of the gland is quite markedly changed the longer the interval after hypophysectomy particularly in relation to the ascorbic acid and the cholesterol content. This goes back to Dr Pincus point about the requirement of certain factors in order for the reactions to proceed.

Rall The fact that certain factors seemed to be required for the reactions to proceed might be the explanation for the longer sustained output when the glands were primed with ascorbic acid. I wonder Dr Pincus whether you have any data on the ascorbic acid content of the glands as they came from the slaughter house. Are they very low?

Pincus Extremely 50 to 75 per cent in a beef gland.

Rall What is the concentration of ascorbic acid in the beef adrenal gland as compared to other species?

Pincus It is not as high as in the rat and perfusion does not bring it to even the lowest level in the rat e.g. about 200 mg per cent.

Long I don't know how the animals Dr Pincus uses are killed but most times they are hit over the head with a sledge hammer and perhaps have their throats cut as well.

Rall This constitutes considerable stress.

Bush I could answer Dr Long's question in part. The cholesterol contents have been done in other experiments by Stack Dunne and the A.W. factor maintains cholesterol at high or normal levels. Dr Renford's preparation which is very similar also maintains the ascorbic acid and the cholesterol at normal levels.

Sayers In the untreated hypophysectomized rat the concentration of cholesterol remains high. As a matter of fact it is higher than normal. The ascorbic acid concentration falls.

Vogt My information is probably not up to date but last spring when Stack Dunne (20) was reporting some of these results he was not quite sure whether the adrenal weight factor was different from the growth factor. Is that point now settled?

Bush I think this is still a debatable point.

the end product that we measure requires certain co factors and that these are perhaps exhausted during the period when the gland is out of the animal and is not being perfused. These co factors then are restored by the priming blood.

Bush Are you still using mainly female glands?

Pincus We are using mostly cow glands, but we have used steer glands for the same experiment.

Bush Priming is required with the female glands?

Pincus Yes but it is my impression that the steer gland requires it even more than the cow gland. Generally we get much poorer results with steer gland and I think it is essentially the situation you are talking about here that it is a phenomenon of disuse. The unavailability of certain substrates may in the steer gland keep it inactive for certain enzymatic transformations.

Selye As I was listening to Dr. Bush the possibility occurred to me that the experiments could be explained without assuming the existence of several ACTHs. Assuming that the weight factor is absorbed more rapidly and has a more transitory effect after each injection its influence upon adrenal weight may be cumulative nevertheless because the effect of each injection (hypertrophy) takes some time to vanish. On the other hand the ascorbic acid factor would presumably have a prolonged and constant action only if injected in beeswax. Could a dissociation of the two effects when the results of these two preparations are compared be due merely to differences in absorption rate? If so we would not have to assume two qualitatively different compounds.

Bush I am afraid I did not emphasize one of the points which is in keeping with the absorption criticism because I was trying to compare this mainly with Dr. Pincus' work. In addition to those groups in Table I we did give the adrenal weight factor in beeswax. The adrenal weight factor and the ascorbic acid factor were given twice a day subcutaneously and also they were given in beeswax intraperitoneally. The results were essentially the same. Again there was no maintenance of the ability of the adrenal to respond to the ascorbic acid factor.

One criticism that still could be made on the basis of difference in absorption rate is that it might be supposed that the adrenal weight factor was the same essential active part of the ACTH molecule. It might be an A.A. factor molecule hooked onto a much larger molecule but in order to act it must be free of that larger molecule. Even though we were doing our best to get over the absorption problem by injecting the material intravenously at various times our experiments may not have been carried out for a sufficient length of time to enable dissociation of the molecule. In other words the second criticism which the absorption rate critics might examine is that the material could not only

injected it failed to cause significant damage although it did cause some hypertrophy of the renal tubules and some degenerative changes in the heart. Similar doses of LAP caused severe damage to the hearts and kidneys of nonadrenalectomized, sensitized rats. These results are in confirmation of earlier observations by Dr Selye. However when our adrenalectomized sensitized rats were maintained on larger doses of adrenal cortical extract the incidence and severity of damage to the hearts and kidneys was greater in animals given LAP than in rats which did not receive it. Some damage occurred in rats maintained on adrenal cortical extract alone. Our data suggest but do not prove that LAP had an extra adrenal damaging effect. It is our hypothesis that rats given cortical extract in amounts just adequate to sustain eucorticalism under nonstress conditions are made adrenally insufficient by the injection of the highly toxic LAP. If LAP has an extra adrenal sclerogenic effect it may be masked in the adrenally insufficient animal. But it is also clear that an excess of adrenal cortical extract has a sclerogenic effect in the sensitized rat.

Selye Since this is a rather fundamental point perhaps I may be allowed to refer back to Figure 1. As delineated there the stress producing agent acts upon one part of the body which we call a target area. This stimulates ACTH production. The ACTH produces an antiphlogistic (or glucocorticoid) action and a prophlogistic (or mineralocorticoid) action through the adrenal cortex. STH synergizes the effects of the prophlogistic corticoids in the target area itself. If an excessive amount of inflammatory tissue develops for instance in an arthritic joint I would not think from the evidence available to us that any type of hormone or hormone combination produces that inflammatory tissue but that stressor agents let us say formalin microbes or allergens produce it. However the degree of inflammation is regulated by pituitary and adrenal hormones in the periphery. Here ACTH certainly acts through the adrenal. STH certainly acts directly in the periphery. However a third possibility exists namely that STH may so alter the effect of ACTH upon the adrenal that an increased prophlogistic corticoid formation results but for that we have no evidence.

Thorn Do you imply that the reason there is no ulceration in the tissue is that STH has no effect in the absence of disease?

Selye What the graph is supposed to indicate is that a stressor agent let us say an allergen or formalin in our formalin arthritis test acts upon one part of the body which we call a target area. In a formalin arthritis it would be the joint tissue. Its action upon the connective tissue of the directly treated joint is quite independent of both the adrenal and the pituitary because it will occur even in an adrenalectomized or hypophysectomized animal. However the degree of the re

Long Has anybody any further matter arising out of the introduction Dr Selye gave to us this morning on the mechanisms by which the adrenal cortex produces different effects?

Sayers I should like to ask if Dr Selye has any data on the concentrations of electrolytes in the plasma of animals given combinations of cortisone and growth hormone?

Selye We have not

Astwood Might I ask if Dr Selye has any further evidence, one way or the other, regarding this effect of growth hormone in intensifying the desoxycorticosterone effect whether it is via the adrenal or directly on tissues? In other words does he feel that the growth hormone, acting upon part of the adrenal causes it to secrete a mineralo-corticosteroid?

Selye For certain actions of the growth hormone it has been possible to show they are not adrenal mediated that they do not go through the adrenal. For others this is doubtful. For instance the growth effect of the growth hormone has long been shown to be direct. The enhancement of inflammation is also a direct effect as judged by experiments on our formalin arthritis test and the anaphylactoid edema of the rat. These effects are manifest even after adrenalectomy. The synergism between STH and DCA as regards inflammation is also manifest after adrenalectomy. However effects upon the kidney may be in a separate category.

The nephrosclerotic effect of growth hormone in suitably sensitized animals has never been seen in adrenalectomized rats indeed so far we have found no combination of cortical hormones which would restore an adrenalectomized animal so that growth hormone would again have this effect. Therefore it is quite conceivable to me that the nephrotoxic effect of growth hormone is mediated through the adrenal and is due to some hormone other than cortisone or DCA since these are not capable of compensating for the loss of the adrenal. Alternately this may be an effect of growth hormone upon the kidney itself a direct one not mediated by the adrenal but one which is dependent upon the simultaneous action of an adrenal hormone. This hormone however is neither cortisone nor DCA.

White What is the action of whole adrenal extract in these experiments?

Selye We have no conclusive evidence in that respect. We did give STH with adrenal extract to adrenalectomized animals but only in nontoxic doses. Perhaps Dr Ingle has something on that.

Ingle We have some rather unsatisfactory data on the relationship of the adrenal cortex to the damaging effects of LAP in the sensitized rat. Our first studies were on adrenalectomized sensitized rats given minimal replacement doses of aqueous adrenal cortical extract. When LAP was

cold can result in nephrosclerosis and hypertension (21) I am not aware of any data bearing directly on the role of the anterior pituitary adrenal cortex axis in causing the damage which results during exposure to cold I suggest that future research might be profitably diverted from studies on excesses and imbalances of exogenous hormones under unnatural conditions to studies of the metabolic and morphologic consequences of exposure to natural stressors under naturally occurring conditions and the role of endocrine organs in these changes However for the sake of speculation let us suppose that it has been proven that exposure to stress causes adaptation diseases in the sensitized organism and that the response fails to occur in the adrenalectomized animal treated with adrenal cortical extract Dr Selye has regarded such evidence as proving that the adrenal cortex mediates the damaging effect of LAP (22)

I have made up a mechanical analogy illustrating a possible relation ship of the adrenal cortical response to the damaging effect of stressors An analogy proves nothing but it may serve to clarify a meaning This particular one is inexact as it was drawn up several years ago but it will serve my purpose This is going to ruin the transactions of the Macy

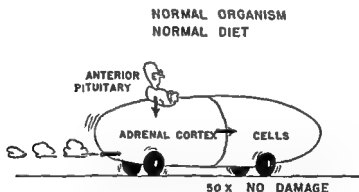


FIGURE 12

Conference Figure 12 represents a normal organism under nonstress conditions There is the adrenal cortex and there is the anterior pituitary which governs it We do not know the basic action of the adrenal cortical hormones so we call it X We can assume with some reason that the cortical hormones affect the rate of some basic process or processes rather than create any new reaction We represent a state of eucorticalism by the arbitrary figure of 50X Under normal conditions adaptation diseases or damage does not occur Moreover a normal organism withstands a considerable amount of stress or load (Figure 13)

sponse is largely subject to the type of pituitary and adrenal reaction that occurs in response to this local stimulation. If we imitate this endogenous hormonal response by giving ACTH, we can inhibit the response to topical stress in connective tissue. The depression in the surface of the skin indicates connective tissue atrophy and lack of responsiveness to topical irritation. For example in the pneumoderma experiment the hydrocortisone treated region was not only unresponsive to inflammation but also atrophic and that is why there was a depression in the skin. Only the prothogistic corticoids such as DCA alone and STH alone and particularly the combination of these hormones (there is more than a summation, there is a potentiation between the two) increase the responsiveness of the tissue to any such prothogistic stressor stimulus.

Thorn This diagram would suggest that STH itself does not antagonize the action of cortisone. I thought that in your experiments it did.

Selye Perhaps I am not expressing myself well. This graph is supposed to indicate that they *do* antagonize each other. Half of the drawing the right half indicates what would happen if A.C. say hydrocortisone or cortisone were predominant. The left half indicates what would happen if P.C. desoxycorticosterone or some other mineralo corticoid and/or STH were predominant. When both of them are present in suitably balanced amounts then nothing happens.

Thorn I may be confused but I still do not understand why you have the arrow indicating STH coming down on one side of the diagram in which there is evidence of proliferation of tissue and STH not coming down on the other side with a tendency toward inhibition of the dip induced by cortisone since it would appear in the adrenalectomized animal that STH can offset to some extent the effect of cortisone.

Selye Let us put it differently then. It may be confusing to illustrate in one diagram, such as Figure 1 what happens when one side predominates. If the target is irritated let us say with an allergen or bacterium or formalin the encapsulation and the fibroplasia in response to this agent would be greatly enhanced.

Ingle I should like to pose a general question which relates to Dr Selye's discussion. Dr Selye has clearly shown that these hormones given in excess or administered to sensitized rats will cause pathologic changes which simulate a number of diseases which occur in man. These facts are important in providing a basis for the making of hypotheses and for further research on the etiology of diseases.

According to Dr Selye's hypothesis derailment of adrenal cortical function as the result of exposure to nonspecific stressors plus certain conditioning factors can cause the adaptation diseases. There are a limited number of data indicating that exposure of the sensitized rat to

UNILATERAL NEPHRECTOMY

HIGH SODIUM, HIGH PROTEIN DIET

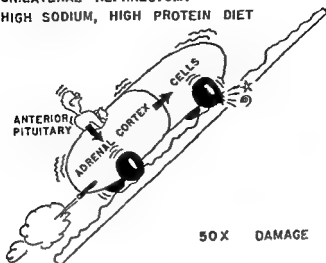


FIGURE 15

suffices to maintain eucorticalism (50X) nothing has derailed or gone awry with its function but the consequence of maintaining that same rate under increased load completes a pattern of circumstances which results in something giving way

It is possible to limit the production of damage by removing the adrenal cortices (Figure 16). The anterior pituitary will still pour out

UNILATERAL NEPHRECTOMY
HIGH SODIUM,
HIGH PROTEIN DIET

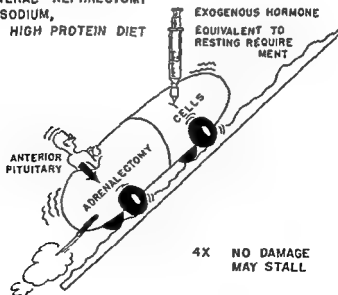


FIGURE 16

Adrenal Cortex

NORMAL ORGANISM
NORMAL DIET

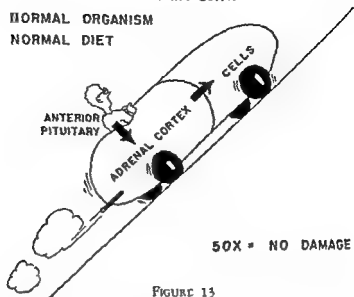


FIGURE 13

without damage although the anterior pituitary must secrete more corticotrophin and the adrenal cortex must secrete more of its hormones to maintain a state of eucorticalism (50X)

Now something is done to the organism to make it more difficult to get along, it is sensitized. We can weaken the capacity of the organism to withstand stress (unilateral nephrectomy) and we can add some obstacles (high sodium chloride). However it can still get along at a normal rate (50X) without damage if it is not subjected to an added stress (Figure 14). When such a sensitized organism is subjected to an added stress (Figure 15) the anterior pituitary must pour out more corticotrophin and the adrenal cortex must secrete more of its hormones. This response of the anterior pituitary adrenal cortex axis only

UNILATERAL NEPHRECTOMY
HIGH SODIUM
HIGH PROTEIN DIET

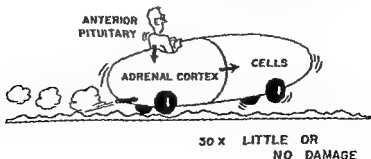


FIGURE 14

There is a difference between conditioning and permissive actions. The difference as I see it and I should like Dr Ingle to correct me if I misinterpret his views is this: in his terminology of the permissive action we assume that one factor say the corticoids are not in themselves doing anything, they are not in themselves pathogenic they merely permit an agent a stressor to become pathogenic. In my interpretation of conditioning factors I assume that an agent in itself is potentially pathogenic but that the conditioning factor can increase or decrease its pathogenic effect. In one case it is only a matter of yes or no the permissive action permits or does not permit. But in the case of conditioning factors there is a matter of degree. The more corticoid that is produced the more corticoid that is exogenously injected into the body the greater the number of lesions which result. That is why I prefer the term conditioning. Permissive to me implies that the corticoid hormones in themselves cause no lesions whatever. Once there is a threshold amount which is just permissive it is assumed that the corticoids cannot do more they cannot intensify a response in proportion to their concentration in blood and tissues.

Ingle That is a possible interpretation but I do not believe that we can exclude the possibility that they are the same. Dr Long brought up the question as to whether a permissive effect is all-or none or whether there is a relationship between the amount of cortical hormones involved and the extent of response. I should like to raise one other point. Although we agree that a combination of factors is essential for the production of damage for the production of disease it is still important for us to find out in the laboratory whether or not the function of the adrenal cortex has to be derailed in order to produce the damage. Is it necessary to have an imbalance in adrenal steroids as between the 11 oxygenated steroids and desoxy like compounds or an imbalance between certain adrenal steroids and growth hormone?

Pincus There is fairly direct evidence that in the human stress causes a differential secretory action of the adrenal. I am referring to the well known urinary steroid pattern that follow stress. For instance immediately following a surgical operation or a burn there is an outpouring of 17 ketosteroids which is rather transient and then a long period in which the output is very low. However rather interestingly we find that the total corticosteroid also shows a rise following stress but continues to stay up. In other words whatever balance 17 keto-steroid represents in the normal nonstressed individual it is certainly changed. I do not think that those fundamental facts are likely to be upset except in detail. I think that the 11-oxy vs 11 desoxy situation is one which will have to be determined by actual analysis. We cannot speculate much on the basis of the indirect data. As far as I know

extra corticotrophin but its target organ is gone. If exogenous cortical hormone is given in an amount equivalent to resting requirements the organism will slow down (4X) or it may die. At the slower rate of living damage does not occur.

What should we call the role of the adrenal cortex in the production of damage in this situation? Is it fair to characterize it as causal? It is a cause of damage in the sense that the adrenal cortical response to stress is a necessary antecedent event but would it not be better to describe the adrenal cortical hormones as having a permissive role in causing damage? Isn't it just as sound if not more so to reverse our terminology here and to represent unilateral nephrectomy and a high sodium load as a part of the pattern of primary causes of the damage? Can we not consider the response of the anterior pituitary-adrenal cortex axis as a conditioning factor?

Selye One aspect of these drawings which Dr Ingle did not comment on obviously represents the G.A.S. the thing that comes out of the exhaust.

I should like to put it this way as far as desoxycorticosterone production is concerned. I do not think it really matters whether the conditioning factors are called *the thing* and the hormone the *conditioning factor* or vice versa. I think that in order to produce certain disease conditions a well defined pathogenic situation is needed. Only if all ingredients of this pathogenic situation are established does disease ensue. Moreover I doubt that one or another ingredient can be considered separately.

For instance in the experiments to which particular reference was made DCA alone did not cause these diseases except if enormous doses were given which might well be considered to be outside of what is likely to happen normally under natural conditions. Sodium chloride will not cause these diseases. DCA will not cause these diseases. Unilateral nephrectomy will not cause these diseases. Any combination of two among these factors will not cause these diseases such as DCA plus salt or DCA plus nephrectomy or nephrectomy plus salt. If all three factors are present then the disease results very easily. I think it is more or less a matter of semantics whether one or the other is called the conditioning factor. If the sodium and the nephrectomy are called the conditioning factors of DCA it is no more justified than if we call DCA plus salt the conditioning factors for the nephrectomy and so on.

I should like however since Dr Ingle has mentioned these factors which mutually synergize each other's effects to say a few words about conditioning versus permissive actions. I missed the early part of the Conference but I understand that this was touched upon then.

or the engine would freeze. But the throttle and not the oil would be regulating the speed of the engine. In the same way if the engine were running at 30 miles per hour and an amount of oil injected which was suitable for 60 miles per hour the engine would begin to smoke and trouble would ensue because of a number of changes in the engine's metabolism.

This concept of adrenal function seems to have a better basis in fact at the moment than one postulating that the adrenal cortical hormones are equivalent to a throttle or a trigger regulating mechanism. Is there any physiologic situation in which it can be proved experimentally that more cortical hormones are injected into the system than are needed relative to the speed at which the engine is going? If not then it means that parts of the body on which the stress is acting are receiving and using all the excess secretions of the adrenal and there is no reason why the heart or the kidneys or any other organ should suffer from an excess of hormones. I think Dr. Sayers put forth much the same argument in his article in *Physiological Reviews* (18).

Thorn: We have observed marked improvement in patients undergoing bilateral adrenalectomy when a very high level of cortisone or compound F was provided throughout the operation and the immediate postoperative period. The dose employed in some of our experiments has been double that which appeared to be necessary for maintenance of a satisfactory clinical condition during and immediately following the operation.

Bush: In other words if an Addisonian was given enough steroid to produce the same urinary increment as in an intact person his passage through the operation could be improved by doubling the dose?

Thorn: Right.

Bush: Under that stress it appears that the normal human turns out if anything less than the optimal amount of cortical hormone?

Thorn: That is true as judged by overall clinical evaluation of the patient's response although we are not yet in a position to make a critical evaluation of the specific effects of large doses of adrenal hormones on aspects of convalescence such as wound healing.

White: Aren't you making the assumption that the tissue responsivity to hormone is the same in the two situations or that the tissue requirements for hormone are the same? Perhaps the level of hormone required to produce a tissue response could differ in the Addisonian as compared to the normal person. I believe this has been shown in normal vs. adrenalectomized rats.

Thorn: There is no doubt that hormone may be utilized differently when first introduced in a patient with Addison's disease. However I doubt whether there is any significant difference in the handling of

there is no direct analysis which would give an answer

Selye Concerning Dr Ingle's question about the derailments of the adaptation syndrome, I should like to clarify my point of view. If I use the term derailment of adaptive hormone mechanisms, I do not mean to imply that this is always and necessarily due to a disproportion between pro- and anti-phlogistic hormones. The term is used rather to denote any deviation in the hormonal response of the organism during stress which causes or permits the development of disease. Looking at Figure 1 which is designed to illustrate the simplest formulation of the G A S and the diseases of adaptation concept, it can be seen that there are three major components: (a) the stressor agent which is of course quite independent of the hormones and usually comes from the outside; (b) the anti-phlogistic hormones, and (c) the pro-phlogistic hormones. Any disproportion between these three factors could have a pathogenic result. For instance, if our topical irritation arthritis experiments are used as a basis for discussion, the introduction of formalin into the joint region corresponds to the action of the stressor. It will cause inflammation with or without the help of endocrine glands by a direct effect upon the target area. However, the course and intensity of the resulting experimental arthritis will be enormously influenced by hypophysectomy, adrenalectomy or treatment with hypophyseal and/or corticoid hormone. If formalin is injected into the joint region of an adrenalectomized animal, the resulting arthritis is much more severe than it would be normally, but its intensity can be artificially repressed by simultaneous treatment with anti-phlogistic hormone (e.g. cortisone). The more cortisone given, the more effectively can the inflammation be suppressed. This is therefore not a yes or no effect. It is definitely a matter of a perfect balance between the pathogen (in this case formalin) and the hormones which are needed to diminish its pathogenic action. It would be entirely wrong to consider that the term diseases of adaptation necessarily implies a derangement in the balance of mineralo- and glucocorticoids.

Bush Isn't the question actually one of whether in the normal situation there is ever a secretion of the adrenal cortex which is greater than that required by the particular stress? What Dr Ingle and also Dr Engel have shown is that in most situations where metabolic responses occur to stressful situations in intact animals, the responses will still occur in adrenalectomized animals if they are maintained on a constant intake of exogenous cortical hormone, but that the extent of the response is limited by the quantity. In other words, there is a quantitative relation. It is rather like the situation which would obtain if an automobile engine had a separate injection system for the oil. If the engine was run at 60 miles per hour, more oil would have to be injected

tuberculosis the dilemma occurs that if his adrenals produce enough antiphlogistic corticoids to suppress the arthritis his pulmonary tuberculosis is likely to be aggravated because here too inflammation and encapsulation will be suppressed. While in the case of rheumatoid arthritis the suppression of the defensive inflammation is beneficial and does not appear to enhance the spreading of the original causative pathogen the reverse is true in the case of the pulmonary phthisis of the same patient. It is hardly conceivable that the adrenals could produce just the right amount of glucocorticoids since no amount is just right for both lesions. Presumably topical conditioning factors must enter the picture in order to decondition the glucocorticoid effects in one place while enhancing them in another.

Long: Aren't we coming to the key point in this whole discussion of your concept that the hormone blood levels as a result of a response to whatever causes rheumatoid arthritis would rise to the value at which it has been shown the spread of tuberculosis would be enhanced? Isn't that the point of Dr. Bush's argument too that actually levels of blood hormone which may be necessary or are required in the damaged area have a pathological effect in the undamaged areas?

Selye: I would agree.

Long: Dr. Sayers' point I think would be that this is where the self-imposed regulatory mechanism would come into play and thus prevent the blood level from being increased to these extremes or at least to the point where tissues are damaged.

Selye: Although this may not be considered to be a separate disease, accidental thymic involution is a condition whose mechanism we have studied in some detail. As it is a well known syndrome in pediatrics we might perhaps use it as an example. Thymus involution during the alarm reaction is certainly due to adrenocortical hyperactivity. It can be produced by any stressor during an alarm reaction; it cannot be produced after adrenalectomy by any alarming agent and finally it can be elicited even in the absence of both adrenals and of stress by minute doses of glucocorticoids. This thymus involution is accompanied by the characteristic hematologic changes of stress as a result of exposure to say cold formalin infections or any other stressor. In this case the corticoids are produced presumably in order to enhance resistance to the general damaging effects of the stressor. However inescapably the thymus responds with dissolution of its lymphoid elements which here represents an unavoidable side reaction of endogenous corticoid overproduction.

The same could be said about inflammation. An alarm reaction inhibits inflammation no matter what is used to elicit the latter. Undoubtedly the production of antiphlogistic hormones during the alarm

hormone in the Addisonian who has been treated with cortisone over a long period of time. Furthermore our bilateral adrenalectomized cases have their own adrenal glands up to the moment of operation.

Ralli Do these Addisonians get more salt than a normal subject?

Thorn We do not use supplementary salt in the maintenance of most of our patients with Addison's disease. They eat a perfectly normal diet.

Long Hasn't it been shown that an adrenalectomized animal subjected to stress actually requires a great deal more hormone in order to live? There must be many examples of that in which the amount of hormone which is required for life maintenance under basal conditions is greatly increased if the animal is placed in an unfavorable environment. That does not say, following Dr. Bush's point, that when the secretion of hormone is increased four or five times the animal is subjected to an excess of the hormone.

Bush Exactly. We know that ten or twenty times as much cortical hormone is required to maintain the rat's work output from a stimulated muscle as is required to maintain its life. However, the point is, does the adrenal under normal stimulation by the pituitary ever in fact put out fifty times as much instead of the required twenty times as much?

Selye When we want to apply this thought to the concept of the diseases of adaptation, I think we ought to take into consideration also that both an excessive or inadequate adrenocortical response may become pathogenic. If during stress the adrenal cortex did not respond with the optimum amount of corticoid production, this in itself would also lead to a pathogenic situation. The corticoid production mechanism would be derailed in the sense that it was inefficient and thus a factor in the causation of disease.

If I might apply that to the much discussed problem of the pathogenesis of rheumatoid arthritis, I would say this. Although we do not know what pathogen (microbe, allergen, etc.) is originally responsible for the production of rheumatoid arthritis, it is evident that the adrenal of a patient who suffers from this disease does not produce the necessary amount of corticoids to prevent the arthritic lesions from becoming manifest. The arthritis is not due in this case to an unresponsiveness of the joints, because if exogenous corticoids are supplied, then the joint lesions improve.

Another point which I think we must take into consideration is this. In the schematic drawing of Figure 1, it has been assumed for simplicity's sake that there is only one target area subject to attack by a single stressor. In clinical medicine this is rarely the case. If one imagines the situation of a patient who suffers simultaneously from rheumatoid arthritis and a well-controlled latent focus of pulmonary

ditions the typical nephrosclerotic corticoid overdosage picture ensues. However, as I said before, I cannot quote any clinical case in which this relationship has been definitely proven.

Sayers Isn't it true that the concept of diseases of adaptation is very difficult to prove or disprove? I do not think it profitable at the present time to continue to discuss the concept at length because of the indirect nature of the evidence. One can come up with almost any answer depending upon the way the facts are juggled. They can be fitted into almost any preconceived concept. Advances in methodology in particular, cortical steroid analysis in peripheral blood, not preoccupation with a concept, will help clarify our understanding of the role of the adrenal cortex in disease.

Pincus I should like to second the motion very heartily. We have been trying, in the case of rheumatoid arthritis particularly, to get some indication of the nature of adrenal corticosteroid secretion, and it all depends on how the measurements are done. If the total corticosteroid output from rheumatoid arthritics is measured, there is no difference from that of normal subjects. The average output for persons of comparable age is 4.5 mg. formaldehydogenic steroids per day in a group of normal and arthritic subjects. However, a detailed analysis of the corticosteroid contained in the urine is done, we begin to see differences. But we do not know whether these differences are traceable to the arthritis or not. There is a good deal of individual variation in what might be called the urinary corticosteroid pattern, and thus it may be the individual and not necessarily the disease which is responsible for the variations. Therefore, I am heartily in favor of Dr. Sayers' remark. We should have better information of blood levels and of the nature of the corticosteroid secretion before speculating too freely, although—the contrast between the 17 ketosteroids and the corticosteroids following damage does provide some basis for speculation.

I have one other piece of information which is of interest. We have compared the total corticosteroid output of a group of about thirty normal individuals with a slightly smaller group of schizophrenic persons. We found to our surprise that a normal healthy individual shows a very consistent output from day to day with a characteristic output level. The schizophrenic, however, varies markedly. This variation might offer a basis for theorizing, but until one can separate that which is characteristic of the adrenal, I think we are juggling concepts.

Bush On Dr. Sayers' attractive theory of the homeostatic mechanism, one would expect the diseases of adaptation to be impossible unless some very severe derangement of the regulatory system occurred. If Dr. Sayers' theory held, it might be expected that increased cortical secretion could occur without any rise in the blood hormone concentra-

reaction plays a special role in the resistance to inflammation during stress. One can hardly imagine that the suppression of inflammation would not enhance the susceptibility to diseases caused by those microbial agents against which the organism normally defends itself predominantly through inflammation. Therefore I think we must admit that disease can be enhanced or caused both by an increase and by a decrease of corticoid production. Indeed even if the pituitary-adrenal system is not abnormal and responds during stress as it should, there remains the original dilemma that the body may be the bearer of two disease conditions, one of which requires an increase in corticoid production and the other of which is aggravated by this same hormonal change. In that case a perfect endocrine response becomes impossible and simultaneous adjustment to the two types of hormonal requirements can only occur as a consequence of some topical compensatory responses perhaps in the sense of selective conditioning.

Astwood: Dr. Bush's question is still not completely answered without a concrete example. It is a theoretical possibility, but are there any combinations of two diseases where the one is benefited and the other made worse by stress?

Selye: Offhand I can only think of the thymolymphatic atrophy, and you may argue that that is not strictly speaking a disease.

Thorn: Perhaps a patient with peptic ulcer and rheumatoid arthritis would present the combination you want.

Selye: It would be extremely difficult to prove in any patient, for instance, one with a peptic ulcer and rheumatoid arthritis or tuberculosis and rheumatoid arthritis, that an aggravation of the lesion known to be normally aggravated by excess glucocorticoids was actually caused in this particular case by the defensive production of such hormones. The so-called Cushingoid changes which have been observed in certain cases with chronic infections, in prolonged aspirin overdosage, in pregnancy and so forth, might be relevant, but I certainly cannot give any particular instance of a patient in whom this was proven.

Long: As was remarked, the urinary excretion of adrenal cortical hormones continues high for a long period of time. After burns, for perhaps two weeks, the level of adrenal cortical steroids in the urine is much higher than normal. It is quite significant that in that period an excess of adrenal cortical hormones, if it represents an excess, is apparently not associated with any deleterious effect such as hypertension or nephrosclerosis.

Selye: In experimental animals, nephrosclerosis caused by exposure to cold has been ascribed to excessive corticoid production. The increase in circulating corticoids is useful to defend the animal against the general damaging effects of cold, but under suitable experimental con-

the other was not In the opinion of the clinicians the normal group was quite healthy

Long Were these steroids that you determined free? They were not conjugated?

Bush They were free

Selye If the self regulating mechanism of corticoid production functioned perfectly during stress then I admit we could never expect any disease to be due to a derailment of adrenocortical hormone production because by definition this would always have to remain the same Certainly under these conditions no lesion due to an excess of circulating corticoids could be expected since such could never occur because of the perfect functioning of the feedback mechanism However, when we first analyzed the mechanism of this phenomenon which we called compensatory adrenal atrophy we pointed out that it is precisely during the alarm reaction that it fails (25) If this self regulation mechanism did function during stress not only diseases of adaptation but the entire adaptation syndrome concept would have to be completely revised One could never expect any increase in corticoid production to occur either to combat stress or to produce disease during stress Even the adrenocortical hyperfunction of the adaptation syndrome could not really take place if the self regulation were always absolutely perfect and I do not think Dr Sayers wanted to postulate that

Long He has not said that

Bush The question is whether or not there are situations in which the pathologic requirements of some stressed target area are such that there is a consistently elevated hormone level in the peripheral blood

Selye To give just one example there would not be thymic atrophy in the presence of the adrenals during stress if there were not an increased blood corticoid level

Bush I quite agree with you but as I said the only examples we know either by direct or indirect means are cases where the anti-phlogistic hormones are elevated

Sayers Dr Selye I would not be quite as dogmatic about the thymic atrophy being due to hypercorticism because I always keep in mind Dr Ingle's experiment showing that in an adrenalectomized animal given a maintenance dose of cortical hormone and subjected to the stress of a fracture typical catabolic reactions occur

Dr Ingle have you noticed thymic atrophy in adrenalectomized animals given a maintenance dose of cortical hormone and subjected to stress?

Ingle We have not studied thymic response under such conditions but I believe that Dr Selye has done so

Selye Yes In an adrenalectomized animal maintained on a certain

tion In other words, the utilization rate of some particular tissue which required more cortical hormone would be increased to such an extent that the blood level of cortical hormone remained close to the normal level with the result that ACTH secretion could be maintained whereas, if Dr Sayers theory held absolutely in the event that the blood level rose there would not be a maintained increase in adrenal secretion

Pregnancy seems to involve such an actual increase in the blood concentration of cortical hormone Fifteen cases studied with Professor Pickering at St Mary's Hospital, London have shown blood concentrations of 17 hydroxycorticosterone in the range of those found in Cushing's disease Thus the increased rate of adrenocortical secretion in pregnancy indicated by clinical findings and estimations of urinary steroids is also associated with an increased concentration of adrenocortical hormone in the peripheral venous blood

Another case is Dr Nelson's (23) experience with patients with fevers of one sort or another in which compound F in the peripheral blood was measured It was found that the concentration of compound F like steroids in the peripheral blood was normal or slightly above normal until the very termination of a febrile disease but in the last two days of the disease the concentration would rise to four or five times normal Here it seems to me is another instance where a response to a pathologic situation results in an increase in the concentration of these steroids in the blood

Long What disease was that?

Bush These were terminal pneumonia cases and severe influenza and things like that

Long In view of Dr Haines (24) recent studies on the presence of these steroids in the placenta have you any idea of how the placenta may be contributing to the situation found in pregnancy? In other words is this high level found in the first second or third trimester?

Bush Most of these cases were in the third trimester

Thorn The selection of patients who are pregnant is interesting but I am not sure that it settles the argument since we have observed an increase in steroids in patients with Addison's disease during pregnancy Presumably as far as the mother is concerned the reaction might not be mediated according to the Sayers theory but from an external source of hormone

Bush In five cases that were followed after delivery the concentration was still high for several days afterwards

Thorn That is very interesting

Ralls Did these patients have perfectly normal kidneys as far as you could ascertain?

Bush I am not a clinician We did two groups one was toxemic and

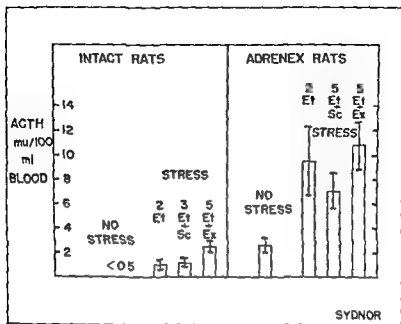


FIGURE 17 Experimental data of Dr Katherine L. Sydnor of the Department of Physiology Western Reserve University School of Medicine. The figure illustrates the effect of stress on the ACTH content of the blood of intact and adrenalectomized rats. Et = ether. Et + Sc = ether plus scald. Et + Ex = ether plus exsanguination. 2, 3, 5 indicate the time in minutes that the samples of blood were removed for analysis following the initiation of the stressful stimuli.

the ACTH you infuse is being very rapidly inactivated and does not accumulate.

Thorn: There is no doubt that a lower dosage level would be possible if the infusion was given over a more prolonged period, but if the infusion was limited to a four hour period 0.25 units per hour was the lowest level at which we could be certain of a statistically significant increase in urinary steroid excretion.

Pincus: That is 250 milli units.

Sayers: But remember the ACTH is probably distributed over the total extracellular fluid volume.

Ether anesthesia for two minutes in rats resulted in ACTH appearing in the blood (Figure 17). It was in very small quantity, but there can be no doubt about its presence. Ether plus scald did not produce a significant increase over ether alone. Ether plus exsanguination seemed to give a higher concentration of ACTH.

amount of cortical hormone the thymic involution can be aggravated by simultaneous exposure to stress. Conversely, even without exposure to stress thymus involution is elicited in adrenalectomized animals by glucocorticoids in proportion to the amount given so that here again there is not a permissive or yes or no effect of the corticoids but they do participate in the production of the lesion in proportion to the amount circulating in the blood.

Sayers Yes that is right. You demonstrated that yourself. In regard to my regulatory concept I refuse to be baited. I have taken my own suggestion to heart. I do not think this is the time for concepts. It is a time for methodology.

If the Chairman so desires I can show a slide of some observations on blood ACTH* which speak against this regulatory concept. Dr. Sydnor has developed a technique for the determination of ACTH in blood (26). It is essentially a modification of Dr. Astwood's oxycellulose technique for the isolation of ACTH from the pituitary. Modifications which Dr. Bartholomew worked out in Dr. Astwood's laboratory have been utilized. The ACTH eluates were assayed in hypophysectomized rats by the adrenal ascorbic acid depletion technique.

The results are presented in Figure 17. The blood of intact nonstressed rats contains no detectable ACTH. The rats were decapitated and bled from the trunk which meant that the pituitary had no opportunity to release ACTH into the circulation. Exsanguination from the abdominal aorta over a period of 2 to 5 minutes resulted in a high titer of ACTH in the blood (Figure 17).

The equivalent of 40 ml. of blood from decapitated rats per assay animal did not induce a significant depletion of adrenal ascorbic acid. Large volumes of blood from nonstressed man and dog did not induce depletion. Man and dog have more blood available than the rat. Under the circumstances the amount of blood lost was relatively small compared to the total blood volume and ACTH discharge was not induced. We estimate the concentration of ACTH in blood to be less than half a milliunit per 100 milliliter. Dr. Thorn, you found that a quarter of a unit of ACTH per hour in man produces an appreciable increase in adrenal activity?

Thorn Yes that gave a reproducible increase in urinary steroids in patients with intact adrenals. It was necessary to continue the infusion of 0.25 units of ACTH per hour over a four hour period however in order to observe this reaction at this dose level.

Sayers That is rather interesting because the half life of exogenously discharged ACTH is about 1 to 2 minutes. Therefore I expect that

*Work supported in part by a grant from the American Cancer Society recommended by the Committee on Growth.

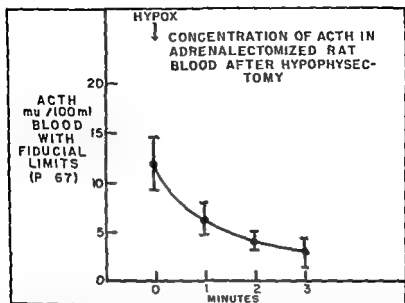


FIGURE 18 Experimental data of Dr Katherine L. Sydnor of the Department of Physiology Western Reserve University School of Medicine. The figure illustrates the rate of disappearance of ACTH from the blood following the removal of the adenohypophysis.

end of one day? In other words, are there extra-adrenal mechanisms for handling ACTH which might be facilitated during a more prolonged survival following adrenalectomy?

Sayers: Yes. I must admit we cannot be sure that when the adrenalectomized rat is stressed, the increased concentration of ACTH is actually due to an increased rate of release from the pituitary. We have not ruled out the factors which you suggest—a change in rate of destruction in the tissues and possibly a change in renal handling of this substance.

Selye: Have you studied changes in pituitary ACTH concentration in adrenalectomized animals exposed to stress?

Sayers: That experiment has not been conducted.

Selye: That would perhaps help to clarify that point.

L: I believe Dr. Gemzell (27) showed that the content is increased *Pincus*. After stress?

L: They haven't studied animals subjected to stress.

Selye: If such a depletion of ACTH stores in the pituitary of an adrenalectomized animal during stress could be shown, that would answer Dr. Thorn's point.

In the nonstressed adrenalectomized rat (decapitated and bled from the trunk so that there was no opportunity for the pituitary to discharge ACTH), the concentration of blood ACTH was relatively high. This would fit with the regulatory concept that removal of the target organ and subsequent reduction in the titer of circulating cortical steroids results in an increase in titer of ACTH presumably because of discharge from the pituitary. However, when the adrenalectomized animals were exposed to ether to ether plus scald to ether plus exsanguination, there occurred a definite increase in the concentration of ACTH in the blood over the value in the nonstressed organism.

Exsanguination of adrenalectomized animals results in a marked reduction of blood volume and the increased titer of ACTH in bled rats could be due to this reduction. However, anesthesia with ether did not appear to reduce blood volume and hence the increased titer of blood ACTH does not appear to be due to simple reduction in the volume of circulating fluid.

Thorn: If a small amount of ACTH is injected into an adrenalectomized rat, do high blood hormone levels result? If so, is this due to inability on the part of the tissues to take up hormone rather than being due entirely to increased secretion of hormone on the part of the adrenal cortex? I should think that the threefold increase in hormone level could be more easily explained on the basis of the target organ being absent rather than on the quantitative relationship to secretion.

Astwood: A very small fraction of the blood would go through the adrenal in two minutes.

Sayers: We have no comparative studies on endogenous ACTH. However, in one experiment injected ACTH disappeared very rapidly from the circulation of the adrenalectomized animal. Unfortunately, we have no studies on disappearance of endogenous ACTH in intact rats with which to make a comparison. I must admit that the type of experiment which you suggest, Dr. Thorn, has to be done before we can get the final answer.

Figure 18 illustrates Dr. Sydnor's work on the disappearance of endogenous ACTH in adrenalectomized animals. These animals were anesthetized, hypophysectomized, and exsanguinated at one, two, and three minutes after removal of the adrenal. ACTH has a remarkably short half-life. From Figure 18, it appears to be between one and two minutes. Adrenalectomized rats were used because they have a very high concentration of ACTH in the blood.

Thorn: How long had these animals been adrenalectomized?

Sayers: Seven days.

Thorn: Do you think the curve would have been different at the

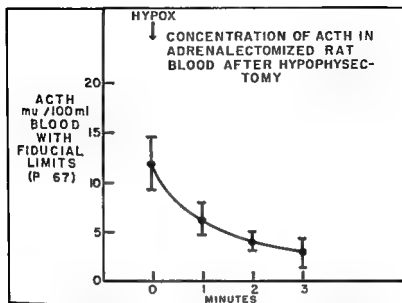


FIGURE 18 Experimental data of Dr Katherine L Sydnor of the Department of Physiology Western Reserve University School of Medicine The figure illustrates the rate of disappearance of ACTH from the blood following the removal of the adenohypophysis

end of one day? In other words are there extra adrenal mechanisms for handling ACTH which might be facilitated during a more prolonged survival following adrenalectomy?

Sayers Yes I must admit we cannot be sure that when the adrenal ectomized rat is stressed the increased concentration of ACTH is actually due to an increased rate of release from the pituitary We have not ruled out the factors which you suggest a change in rate of destruction in the tissues and possibly a change in renal handling of this substance

Selye Have you studied changes in pituitary ACTH concentration in adrenalectomized animals exposed to stress?

Sayers That experiment has not been conducted

Selye That would perhaps help to clarify that point

L1 I believe Dr Gemzell (27) showed that the content is increased

Pincus After stress?

L1 They haven't studied animals subjected to stress

Selye If such a depletion of ACTH stores in the pituitary of an adrenalectomized animal during stress could be shown that would answer Dr Thorn's point

Sayers It would help

Long Didn't you show that when the adrenals were taken out there was an immediate loss?

Sayers Dr Gemzell and Dr Cheng carried out experiments of this type. Twenty-four hours after the adrenals were removed the concentration of ACTH in the pituitary went down to 25 per cent of the normal value. Then it was gradually restored to normal. At twenty days it actually reached values greater than normal.

We have not been too enthused about the determination of pituitary ACTH in chronic experiments, the results are difficult to interpret. However Dr Selye's suggestion is a good one. In acute experiments in which the animals are sacrificed two minutes after the stress is applied an appreciable decrease in the content of ACTH in the pituitary suggests an increased rate of release.

Selye Another argument in favor of an increased rate of release is that if excessive doses of cortisone are administered to animals and the animals exposed to stress during that time the adrenal atrophy normally caused by the cortisone is abolished or at least counteracted by the stress. Under these circumstances the adrenal will enlarge anyway although there is too much cortisone in the body.

Pinchus We have also attempted a study of the ACTH of rat pituitary during the administration of cortisone in doses ranging from 50 μ g to 10 mg per rat per day. No change was seen.

Conn What happens in an adrenalectomized ether stressed animal if hydrocortisone is given intravenously?

Sayers We are interested in this problem. However the rat is not the animal for this study because of the limited quantities of blood. Dr Sydnor plans to conduct studies of this nature in the dog. In the adrenalectomized dog the titer of ACTH is very high. It will be of interest to determine the effect of injected steroids on this high titer.

Rall Were these other experiments that you mentioned done in the rat?

Sayers They were carried out in the rat.

Rall Do you feel that they are a satisfactory index of ACTH activity in the blood?

Sayers It is a question of the reliability and specificity of the method for determination of ACTH in blood. It is an adrenal ascorbic acid depleting method. The recovery is excellent. Dr Sydnor has compared the oxycellulose technique as against direct transfusion in adrenalectomized animals. That is the ACTH titer in the blood of adrenalectomized animals was determined by two methods: the oxycellulose technique and a technique in which the blood from an adrenalectomized rat was collected and immediately transfused into the test animal. The oxycellulose

technique yielded titers of about 80 per cent of those that were obtained by direct transfusion. The oxycellulose technique appears to recover most of the ACTH from blood.

Long: Dr Vogt remarked if I may speak for her that this rapid response in the adrenalectomized animal to ether and ether plus scald looks like the operation of a hypothalamic mechanism.

Sayers: Yes, we have that very much in mind.

Vogt: What I mean to say really is that you should not feel that you are cutting the ground from under your own feet by these experiments. There must be more than one mechanism of regulation.

Sayers: As I said before, I do not think that concepts are in order at this time. The oxycellulose method for the determination of blood ACTH offers new possibilities of understanding the regulation of ACTH discharge. We have been dependent upon indices which measure changes in target organs hours after the pituitary has discharged ACTH. Now we have a technique for measurement of ACTH titer within a few minutes after it has been discharged.

Rall: With the oxycellulose method, do you use whole blood or plasma?

Sayers: Dr Sydnor is using whole blood. A study is being made of the distribution of ACTH between red cells and plasma. Until this distribution is known and until more is known about the stability of blood ACTH, the physiologic work will be confined to analysis of whole blood.

Thorn: If you infuse blood taken from an hypophysectomized rat is any ascorbic acid depletion of the adrenal in the test animal shown?

Sayers: Blood from hypophysectomized rats in a dose equivalent to 18 ml. did not produce a significant degree of depletion. On the other hand, 1.5 ml. of blood from an adrenalectomized animal gave a very significant degree of depletion.

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EXISTENCE, NATURE, AND SITE OF PRODUCTION OF A SALT HORMONE (MINERALO-CORTICOID) SECRETED BY THE ADRENAL GLAND

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IT MIGHT BE A good idea to start this discussion by remembering why it is that doubt has ever arisen particularly in the minds of clinicians about the existence of what we term with Dr Selye mineralo corticoids since it is perfectly obvious that disturbances in the salt metabolism play such an important role in the syndrome of adrenal deficiency. The first reason is that those steroids which are known to be secreted by the adrenal cortex and which are very active in the glycogen deposition test influence the salt particularly the sodium metabolism in the same way as the substance considered to be the prototype of a mineralo corticoid desoxycorticosterone. This has been shown to be true in man and Roberts and Pitts (1) have given figures for the adrenalectomized dog which show that the same sodium retention can be obtained with cortisone and with DCA provided the dose ratio is 20:1 irrespective of the absolute dosage used. I gather that Dr Pincus group in its work on the rat has found the same to be true for certain dosages although they found that if they gave more cortisone the position was different and the salt retention was changed into an increased salt excretion. Thus it is perfectly legitimate to ask whether corticosterone and hydrocortisone the main known constituents of adrenocortical secretion may not fully account for its salt retaining power.

Another reason lies in the fact that for many years desoxycorticosterone was assigned the role of the natural mineralo corticoid. There are still workers who hold that view but there are certain difficulties about it which at least in my opinion make it unacceptable. One of the difficulties lies in the toxic signs so easily produced with DCA and so rarely seen with cortical extracts. It is possible that they are only the result of overdosage and not due to a qualitative difference between natural hormones and DCA but it is not too easy to reconcile the view that DCA is a normal product of adrenal secretion with the discovery by the Worcester Foundation group that the adrenal gland is capable

of 11 hydroxylating I almost feel inclined to say detoxifying DCA. The main difficulty however arises from the observation that according to most authors DCA is not found in adrenal blood and that its yield from adrenal gland tissue is infinitesimal [29 mg from 1000 Kg beef adrenal according to Reichstein and Euw as cited in Thatcher and Hartman (2)]. Admittedly there is one exception to these negative results in the finding by Hechter *et al*, (3) of DCA in cow blood obtained at slaughter and in adrenal blood perfused through a cow adrenal after the addition of ACTH. Two facts however make one wonder whether the DCA does not only occur under very abnormal conditions as a product of incomplete synthesis. First the results concerning DCA are reported not to be consistent and second the content of the blood collected from the vessels of the neck is hardly lower than that of the adrenal perfusate (120 μ g against 140 μ g per 2 l). I hope Dr Pincus will correct me if there has been new evidence on these points.

Pincus You have stated the circumstances exactly. In addition to the experiments that have been published there are some others that Dr Zaffaroni has done and he finds that the increase in desoxycorticosterone following ACTH is very small compared to the increase in every other constituent. In other words whereas there may be somewhere between an eight and ten fold increase in the other constituents there is only a small percentage increase in desoxycorticosterone in the perfused gland.

Sayers What about the peripheral blood?

Pincus It is extremely variable.

Vogt In this connection it is interesting to remember that DCA is not the only substance known to cause salt and water retention and to benefit Addisonians: the same effects can be obtained with glycyrrhetic acid (or its glycoside glycyrrhezinic acid) through a hitherto unknown mode of action (4). Chemically this acid is sufficiently different from steroids to exclude any likelihood of its occurring in the body. The suspicion that its mode of action also differs from that of the corticoids is at present based on only two observations: the retention of salt and water it produces in normal man cannot be duplicated in the rat nor can the beneficial effect observed in Addisonians be obtained in adrenal ectomized rats.

Attempts were made by Bibile and myself (5) to test whether the activity of extracts of adrenal blood in prolonging the life of adrenal ectomized rats kept at low temperature was due to the content of corticoids containing an hydroxyl group in the C₁₁ position. The content in 11 hydroxylated compounds was measured by their capacity to depress the eosinophils in adrenalectomized mice: an effect which is as far as we know restricted to these compounds and is certainly not shown by

DCA The presence in the perfusates of a compound without oxygen in the C₁₁ position but active as is DCA, in prolonging life, might have been shown in a discrepancy between assays of the same extracts of blood on the mouse eosinophils and on the rat survival time. No such discrepancy was found. This may mean either that these biological tests are not accurate enough for the detection of a hormone which might prolong life without acting on the eosinophils or that there is no hormonal activity in adrenal blood which is not accounted for by compounds which are equally potent in depressing the mouse eosinophils. Knowing only too well the great inaccuracy of the biological tests involved I would hesitate to draw any conclusions from negative experiments such as these. Assuming however the tests to be sufficiently accurate to exclude the presence of a substance lacking in effect on the eosinophils would they also exclude the existence of a separate mineralo-corticoid? I think not, because the question may be wrongly put by assuming that the mineralo corticoid we are searching for must be like DCA in all its biological properties, including lack of effect on circulating eosinophils.

It seems to me that the view of the existence of a mineralo corticoid is much easier to defend if one assumes that it is not identical with perhaps not even very nearly related to DCA. And in fact we have now evidence from three sides about the existence of a natural mineralo corticoid that differs from DCA. The older work done on adrenal extracts culminated in the isolation by Thatcher and Hartman (2), of a compound with very little effect on glycogen metabolism but strong sodium retaining properties. Its solubility in water and poor solubility in a number of organic solvents showed that it was decidedly more polar than and obviously not identical with DCA. The first indication of the presence in adrenal blood of a compound with a strong action on salt retention was found in a single experiment by Spencer (6) on the adrenal effluent of the dog. The work was unfortunately not followed up by the author so that the possibility that compounds B and E might have accounted for the activity was not entirely excluded. More recently Tait Simpson and Grundy (7) separated a fraction from adrenal extract which travelled in chromatographic experiments at very nearly the same speed as compound E but showed a salt retaining potency of many times the activity of the cortisone present. There is no doubt that this substance is neither DCA nor 17 OH DCA. The authors in collaboration with Bush (8) succeeded in isolating the same substance from the adrenal effluent of the dog and the monkey so that the fact that it is a secretory product of the adrenal cortex has been ascertained. Table II has been computed from their figures. Shown in it is the mineral activity of steroids which they fractionated on paper

TABLE II

Mineral Activity of Steroids from Perfusate of Dog's Adrenal
Fractions Separated by Paper Chromatography

Region of Compound	Shown by Fluorescence (μg)	Equivalent Mineral Activity (as μg DCA)	Actual Mineral Activity (as μg DCA)
F	225	20	20
E	15	1	56
B	65	10	0.15

Reprinted by permission from Simpson S. A. Tait J. F. and Bush I. E. Secretion of a salt retaining hormone by the mammalian adrenal cortex *Lancet* 2: 226 (1955)

They eluted the regions of the chromatogram which corresponded to compound F to compound E and to compound B. In each of these regions they estimated the amount of corticoids as shown by fluorescence and column 2 gives the figures obtained.

All these compounds have a certain activity on salt retention. The test which Tait and Simpson have used is actually not entirely one of sodium retention. It is the ratio of sodium over potassium in the excreted urine. The mineral activity of the known compounds in terms of DCA is shown in the third column. It can be seen that 225 μg of compound F in the particular adrenal effluent from the dog corresponds to an activity of 20 μg of DCA since F has about one tenth the mineral activity that DCA has. The activity found corresponded very well to this (last column).

The position is entirely different with the eluate of the region to which compound E travels in that the expected mineralo corticoid activity would have been 1 microgram of DCA whereas the activity found was 56 micrograms. Finally in the region to which compound B travels in these chromatograms there is again reasonable agreement between the expected and actual mineral activity. Therefore most of the salt retaining activity of the adrenal effluent of the dog was due to a compound eluted from the region to which compound F travels. About two thirds I should say in this particular experiment of the mineral activity of the whole blood was due to that compound and only the remaining third to known compounds.

W. Hite: How was the actual mineral activity determined?

Vogt: The mineral activity was determined by injecting the eluates from these regions into adrenalectomized mice which had been loaded with a solution of radioactive sodium and potassium and by measuring the ratio of sodium over potassium in the urine.

The chemical identity of this substance is not yet established. We are told that the substance is not an α β unsaturated 3 ketone but that its reaction with triphenyltetrazolium chloride indicates the presence of an α ketol grouping in the 20-21 position (9). If this color reaction may be taken as an index of the quantity present, the biological activity of the compound as measured on the sodium/potassium ratio of urine excreted by adrenalectomized rats is 20 times that of DCA.

I do not know whether Dr. Bush has any more evidence of the possible identity or lack of it of the substance with that isolated by Thatcher and Hartman.

Bush: No.

Vogt: Does it fit so far the theory that they might be the same substance?

Bush: The data of Hartman and Thatcher only give very rough ideas as to its partition coefficients so that one cannot say. However, Dr. Mason* has been doing similar work on the amorphous fraction using the life maintenance assay in which the assay for the different steroids runs very parallel to the mineralo corticoid assay as done by Tait and Simpson in the sodium/potassium ratio. He has some results which correspond with these and it looks as though this compound is responsible for the high life maintenance activity of the amorphous fraction.

Dr. Mason is of the opinion that his substance which so far has exactly the same properties in terms of chromatographic separation and so on does possess an α β unsaturated 3 ketone group but he is not dogmatic about it. I think both Tait and Simpson are doubtful about their statement that it does not contain the group and Dr. Mason is also doubtful about his statement that it does. I do not think that has been cleared up yet.

Vogt: Before I discuss the biological functions that such a compound may fulfill I should like to say a word about attempts at detecting mineralo corticoids in the urine. The findings of Luetscher, Deming and Johnson (10) describe a sodium retaining activity of urinary extracts of edematous patients. That activity dropped suddenly when therapeutic measures provoked a diuresis and rose again when there was a clinical relapse. We do not know whether there is a connection between this substance and that of Simpson and Tait. Even more difficult to analyze are the findings of Stewart, Robson and Tompsett (11) according to which a substance akin in its chemical properties to DCA increases in urine after salt deprivation, after ACTH administration and after surgical procedures. I think at present we can do no more than to register these observations.

As regards the biological effects of mineralo corticoids the only point

*Mason, H. Personal communication.

on which there is complete agreement is that sodium retention is invariably found. Potassium excretion is usually increased but the potassium metabolism is too intimately linked with the carbohydrate metabolism to produce a simple picture valid in all circumstances. It seems certain that the sodium retention has both a renal and an extrarenal component. The renal component was admirably reviewed at last year's Conference by Dr Pitts. We are however left uncertain whether a mineralo corticoid is essential or whether the 11 hydroxylated compounds are adequate to satisfy all hormonal needs of the kidney. There is nevertheless no doubt that DCA is more efficacious than cortisone in preventing renal sodium loss by the adrenalectomized animal.

That there are extrarenal factors involved has been shown by a large number of observations. There is for instance the fact that DCA will protect an adrenalectomized rat from water intoxication even under conditions when there is diminished water excretion so that it is within the tissues that the shift of the water from the intracellular to the extracellular space must take place. Further in the adrenalectomized nephrectomized rat DCA prevents the seizures induced by giving excess water (12). Though comparative figures are not given it is interesting to note that the same authors (13) have also shown that cortisone is more effective than DCA in protecting the intact animal from water intoxication so that here again the physiologic data do not demand the existence of a mineralo corticoid.

Thorn In preventing water intoxication how long before the administration of water is hormone administered? Is the time interval of short enough duration to eliminate a renal effect as the mediating mechanism?

Vogt These are adrenalectomized nephrectomized animals which were kept alive. I think for two days or so.

Thorn But these animals had been treated continuously with desoxy corticosterone before they had been operated on?

Vogt No most animals were treated only after the operation.

Sayers There is an important point here. Desoxycorticosterone acetate has a relatively weak effect or has no effect in protecting these animals against a water load if given just prior to the administration of the water but if desoxycorticosterone acetate is given for a long period of time before the water load then it does have a truly protective effect. Therefore what Dr Thorn brings up is important the time relationships in regard to injection of the hormone and the administration of the water load.

Vogt And then we are confronted by the problem that the desoxy corticosterone if given a long time before might be 11 hydroxylated because apparently it is not only the adrenal which can do that.

Sayers That is one interpretation. Another interpretation of course is that DCA expands extracellular fluid volume and induces a hypernatremia which would provide a protective reserve of sodium in the case of adrenalectomized animals given a water load. Paradoxically they do suffer circulatory collapse when given a water load and are otherwise untreated.

Gellhorn A number of years ago we studied the electroencephalogram under conditions of water intoxication with and without desoxycorticosterone. We found as the expression of protection which you mentioned Dr. Vogt that the cortical changes were much milder in the animals treated with desoxycorticosterone. They consisted in general of slow potentials of a frequency of 1 to 3 per second and an amplitude of 300 microvolts. Convulsive spikes did not appear. However in rats not injected with desoxycorticosterone convulsive spikes appeared singly or in groups. These spikes were commonly preceded by slow waves in the normal animal subjected to water intoxication (14).

Vogt Finally the extrarenal factor is demonstrated by clinical observations on patients with renal damage. In such patients large doses of corticoids raise the serum potassium instead of lowering it. The simplest explanation of this paradoxical phenomenon is that the hormones cause a shift of intracellular potassium into the extracellular fluid just as they help to shift the potassium from the plasma into the urine when the kidney is not diseased. These effects are observed with ACTH and DCA and with high doses of cortisone so that they do not help us much in assigning a special function to any one type of corticoid (15).

Thorn Could you tell us who it was who demonstrated the rise in serum potassium with desoxycorticosterone? There have been one or two American observers who have suggested quite the contrary—that is, that it is possible to lower serum potassium in the presence of renal disease with desoxycorticosterone. We have not been able to confirm this.

Vogt This was reviewed by Luetscher in the Ciba Conference published in 1952. It may not be his own work as he was opening a discussion.

Sayers In the nephrectomized rat both DCA and cortisone increase the rate of passage of potassium into the gut lumen. Despite the fact that the cortisone was probably mobilizing potassium from tissues in the nephrectomized animal the plasma concentration of potassium was either equal to or less than the control nephrectomized animals which were not given cortisone.

Vogt That would be different from man.

Sayers This is an extrarenal mechanism involving the gastrointestinal tract.

Thorn This is apparently comparable to observations made in man Dr Steele and his group in New York and Dr Kendall Emerson in our group using resin exchange in man and in patients with Addison's disease have indicated that when the sodium content of the stool is increased with resin it is possible to demonstrate that desoxycorticosterone is followed by increased potassium loss in the stool and reduced sodium excretion

Vogt The idea that the actions of cortical hormones are intimately linked with the energy supply required for work performance by all organs of the body has appealed to many workers in the field Tentatively I should like to suggest that the mode of action of these hormones on salt metabolism is equally exerted through an effect on the energy supply to cells renal or extrarenal which perform osmotic work Such a concept does not in any way prejudice the possibility that some corticoids may act predominantly on ion exchange mechanisms and thus constitute what have been termed mineralo-corticoids

The last point I should like to raise concerns the site of production of mineralo-corticoids within the adrenal cortex You are all familiar with the hypothesis put forward by Swann (16) which suggests the zona glomerulosa as the site of production of the salt hormone Many authors (17 18 19 20) have reported atrophic change in the glomerulosa when excess sodium was fed to rats and hyperplasia when a sodium deficient diet was fed to rats This finding favors Swann's hypothesis but alternative explanations are possible Differential response to environmental conditions of the various cortical zones is not restricted to changes in the salt content in the diet Thus a high dose of estrogen administered to rats for 5 days will deplete the fasciculata of lipids but leave the lipids of the glomerulosa untouched (21) This would also appear to confirm Swann's theory but the further observation is more difficult to interpret if treatment with hexestrol is continued for two or more weeks the lipids also disappear from the zona glomerulosa Though it is I think a sound biological principle to assume that parts of an organ which show such striking differences in their histochemical responses are endowed with different functions it may be an oversimplification to assume that the task of the different layers is the production of different substances What appears quite certain is the fact recently confirmed by Miller (22) that the responses of the zona glomerulosa are not as are those of the zona fasciculata dependent on the secretion of ACTH The salt metabolism is generally thought to be less dependent on the pituitary than the carbohydrate metabolism a fact which would favor the conception of the zona glomerulosa as a site of production of salt hormone Whether however this is true in the human is anything but certain Indeed many authors have observed that ACTH has an

influence on mineral metabolism in man. To cite only some examples Conn, Louis Johnston and Johnson (23) have shown that ACTH has the same effect as DCA on the electrolytes of thermal sweat and this has been confirmed by Locke, Talbot, Jones and Worcester (24) with a different technique. These authors also demonstrated that cortisone has no such effect. Taking this index as a measure of mineralo corticoid activity the conclusion would be that in man at least the secretion of mineralo corticoids cannot be restricted to a part of the cortex which functions independently of the anterior lobe. It may therefore be that the difference between the zones does not lie or does not only lie in the type of hormone produced but in the nature and perhaps the duration of the stimulus required for their activation.

Another problem if we accept Swann's hypothesis is posed by experiments on adrenals allowed to regenerate after complete enucleation. Although the regeneration takes place from the capsule and one might expect glomerulosa tissue to be formed there is no doubt that the regenerating gland produces hormones assigned by Swann's hypothesis to the zona fasciculata. Moreover this occurs at a time when the serum sodium is still below normal (25). Perhaps a decision for or against Swann's theory should be withheld until instead of using DCA as was hitherto done in order to inhibit the activity of the zona glomerulosa by administering its presumptive hormone this type of experiment can be repeated with the substance isolated by Simpson and Tait. One serious difficulty in the interpretation of the histologic picture lies in the fact that the histologists tell us that there is no cytologic criterion by which to distinguish a glomerulosa from a fasciculata cell; the identification of the zones lies mainly in the arrangement of their cells and possibly in histochemical reactions which are reliable only in undisturbed normal glands (e.g. the lack of ascorbic acid in the zona glomerulosa). Quite obviously these problems are now ripe for direct experimental attack by more attempts at the chemical identification of the products of adrenocortical secretion. The examination should be carried out in the living animal subjected to procedures making excessive or minimal demands on the processes regulating the salt balance.

Bush I think there are a few loopholes in the argument. So far none of the work that has been done has actually proved that the material is a steroid. The supposition that the material is a steroid is the most likely and the most economical hypothesis at the moment but it is not proven and it is only on the example of William of Occam that we adopt that hypothesis¹. However if we assume that the substance is a steroid then the chemical tests and the position on the chromatogram and the other properties which have been worked out so far would indicate that it has an α ketol side-chain and that it has two esterifiable

hydroxyl groups. It is unstable to dilute bicarbonate solution. It is extremely unstable also to dilute solutions of strong acid and the only way it has been possible to recover activity after acetylation has been to hydrolyze it by what at present is an unknown enzyme. It is merely a simple esterase preparation which Tait and Simpson make up from washed red cells.

Sayers: Doesn't that seem a bit unusual? You would expect the acetate to be hydrolyzed in the test animal.

Bush: You would. I agree, but that is what has been found so far. On the other hand, the assays are done with rats while the red cells come from rabbits, so that one is not bound to assume that the stuff is hydrolyzed. Also, it may not be hydrolyzed at a fast enough rate to be active. The polarity is such that if a prolonged paper chromatogram is done, it can be separated from cortisone itself; it is less polar than cortisone. In other words, if a Zaffaroni type of chromatogram is run for 24 to 48 hours, then the material is not separated at all from the cortisone. If the chromatogram is run for a week until the material is right down to the front, this spot is just in front of cortisone itself.

They have also now several materials from Reichstein and have checked on all his substances which would be expected to flow in that part of the chromatogram; none of them are identical with it. That includes substance P and substance R, which I think are the 3 hydroxy analogues of Dr. Kendall's compound II, and Prof. Reichstein's substance S. Isn't that correct?

Kendall: I forget.

Bush: Perhaps these results should first be compared with Dr. Wintersteiner's results (26). Or did you also, Dr. Kendall, find that the amorphous fraction was labile to dilute bicarbonate solution?

Kendall: No, we have not shown that. All steroids with the ketol side chain are labile in the presence of air and dilute alkali.

Bush: Except that the acetates of E and F can be hydrolyzed with methanolic bicarbonate and the material recovered as a free compound?

Kendall: In the absence of oxygen.

Bush: Dr. Wintersteiner compared whatever he called his compound at that stage with his amorphous fraction, and he found that with the amorphous fraction under identical conditions with bicarbonate the activity was lost. However, the great instability of his amorphous fraction is comparable to that of Tait and Simpson's substance.

Dr. Mason has recently been doing similar work using life maintenance as an assay, and has also found by the Zaffaroni type of chromatogram the material which he had been hunting for in the amorphous fraction running with cortisone; it does appear to have an α , β unsaturated ketone group. Tait and Simpson say they cannot detect an

α β unsaturated ketone group but they are not yet sure of this. The structure is extremely dubious and, as I say, all these speculations are based on the assumption that it is a steroid.

White I take it that the possibility of a compound with α β unsaturation is pretty well eliminated?

Bush I do not know. Tait and Simpson have done this work largely on the basis of material extracted from glands. They have tested it pretty thoroughly, however, I do not think it is all that clear.

Vogt Is this substance stable in organic solvents provided it is protected from air?

Bush Yes. In the extractions from blood I did not use any more rigorous precautions than I usually use for compound I, compound B and others like that.

Long Is the amount in blood increased by the administration of ACTH?

Bush That is what we are starting to determine.

Thorn Can you explain why this compound does not manifest itself in the usual adrenal extracts? I mean that salt retention with the potent commercial adrenal extracts available has never been a very striking feature in patients with Addison's disease.

Kendall It is there.

Thorn I thought you stated there is more of the compound in the amorphous fraction.

Kendall Yes, but that is in the extract.

Thorn If it is in the extract, salt retention is not very striking with the most potent adrenal cortical extracts available. Do you feel that perhaps the action of a salt retaining substance is buffered by the other compounds present in the extract?

Bush Tait and Simpson have done it with their assay and found that all these compounds, including E, F, B, A, DCA, and this substance, have an additive effect on the sodium-potassium ratio. That is not to say that they necessarily have an additive effect on sodium chloride excretion.

Thorn No, that is different.

Bush But as regards the sodium-potassium ratio, they are almost exactly additive in their effects.

Thorn It has been known for years that excessive sodium retention is rarely observed in patients with Addison's disease treated even with large doses of adrenal cortical extract. This is quite in contrast with the effect observed with desoxycorticosterone.

Bush Dr. Kendall and Dr. B. H. Wells showed a long time ago that if doses of the amorphous fraction were given which were equivalent to toxic doses of desoxycorticosterone in terms of life maintenance assay, there was never excessive salt retention.

Kendall Salt retention is raised to the normal level but not above

Sayers Dr Bush can you account for the salt retaining properties of adrenal cortical extract on the basis of the activity of, let us say the F like steroids and this steroid which is very active in sodium retention?

Bush Yes There is one thing which Dr Vogt did not emphasize and that is that in the dog experiment we also assayed all the other regions of the chromatogram including the origin and down to the front where desoxycorticosterone would have been found if it had been there The activity over all of the rest of the chromatogram was less than a 0.5 microgram equivalent of desoxycorticosterone per centimeter It would be somewhere less than a microgram per 3 or 4 centimeters of the chromatogram out of a total chromatogram of 45 centimeters

Tait and Simpson also showed that the same thing holds true for adrenal extract namely that the sum of activities obtained from the known steroid and this compound after fractionation on a chromatogram adds up to the total activity of unfractionated adrenal extracts Therefore there is no need to postulate any other highly active mineralo corticoid in adrenal extract or in the adrenal blood experiments we have done so far

Sayers No but as I see it the problem that must be answered is the one which Dr Thorn posed why is there not excessive sodium retention when large doses of adrenal cortical extract are given?

In some work that Dr Woodbury and I carried out a few years ago we were interested in the problem of potentiation or antagonism of cortisone and DCA We did find antagonism between cortisone and DCA when the animals were given a sodium load The hypernatremia in animals given the combination of DCA and cortisone was less than in animals given DCA alone I want to emphasize that the dose of cortisone in these experiments was relatively small

Vogt You are talking though of animals which have their adrenals is that right?

Sayers Yes these animals had intact adrenals which complicates the picture

Vogt These assays are done entirely on adrenalectomized animals In them salt retention occurs readily whereas it is much more difficult to get salt retention by whatever means in an intact animal

Sayers I realize that this is a complicated problem A patient with Addison's disease has difficulty not only in a situation where he is deprived of salt but also in a situation in which he is given a sodium load This is something we should keep in mind in an understanding of the whole problem that is for the over all picture of the combination of steroids and their effect on electrolytes

Rall There is also the point that Dr Pitts made last year which was

■ β unsaturated ketone group but they are not yet sure of this. The structure is extremely dubious and as I say all these speculations are based on the assumption that it is a steroid.

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order to determine its chemical properties. At the moment these speculations are based on amounts which even he would hesitate to use for chemical work.

Li Is it the same compound that Leutscher at Stanford reported in the urine?

Vogt That is what we do not know.

Bush It is quite possible. There are several groups in Germany which have been working on this matter but they have all been using the benzene fractions from benzene water partitions of urine extracts.

Selje You are thinking of Weissbecker and Staudinger.

Bush That is right.

Vogt There is no evidence that their substance comes from the adrenal and there is no evidence that it varies if either ACTH is given or if the subject is under different conditions. The only criterion is that chemically and in its solubility it behaves like desoxycorticosterone acetate. I think this is really very difficult to interpret.

Bush You saved me from having to be rude. However the thing about that work as you have emphasized is the elementary supposition that desoxycorticosterone is the mineralo-corticoid secreted and that it will be in the benzene fractions. All the past attempts to detect mineralo corticoids in urine by taking benzene fractions after benzene water partition automatically have missed this compound because the material has a polarity similar to the gluco-corticoids and would not be separated from them by simple benzene water partition.

Long Have you made an estimate in the adrenal venous material of the proportion of this material as compared to the other steroids?

Bush If you will allow me our economical assumption. I worked it out and it is rather less than one part of this substance to 100 parts of compound F in the dog.

White If one more if can be postulated namely if this compound is a steroid in view of its chemical behavior and polarity. I wonder whether Simpson and Tait have had an opportunity to test either compound V or the compound N of Reichstein?

Bush Compound V?

White It is the allopregnane tetrol namely allopregnane 3(β) 11 (β) 17 α 21 tetrol 20 one.

Bush Related to E or F?

White It is related to F it has an 11 hydroxyl group.

Bush I think they have tested substance N. I do not think they have tested the substance related to 17 hydroxycorticosterone.

White In any event I do not think the polarity data would fit.

Bush Oh no it couldn't be. Actually this flows behind 17 hydroxycorticosterone. They have bioassayed compounds N, R, and P which I

that adrenalectomized animals have considerable difficulty in handling acid loads. It seems hard to separate these various functions of the kidney into the handling of sodium or potassium or some other fraction and it would seem that there was some over all disturbance in the absence of the adrenal gland as far as the kidney is concerned.

Sayers: It would be very interesting, it seems to me, when you are able to isolate more of this factor which causes sodium retention to study its effect on sodium retention under conditions of sodium loading.

Vogt: There is no evidence that this substance causes sodium retention in the normal animal. That is important.

Long: It is different from DCA then.

Vogt: I would not say it does not but so far there is no evidence. That I think is a point we have to be careful about when drawing conclusions.

Thorn: That may be merely quantitative.

Vogt: It may be.

Thorn: In man one can demonstrate with certain doses of compounds E and F under certain circumstances increased excretion of sodium and in other circumstances slight sodium retention. However with large doses of these substances intravenously as a continuous infusion there may be tremendous sodium retention equivalent in every way to that observed with desoxycorticosterone.

Bush: Yes but Drs. Kendall and Wells did try with something like twenty times the equivalent dose of desoxycorticosterone and found that it would not raise the serum sodium concentration of the adrenal ectomized dog above the normal level whereas of course at that dose desoxycorticosterone would have killed the dog quite rapidly.

Long: Did Hartman show sodium retention in the blood & with his so called sodium factor or & as it only in the urine?

Kendall: I think it was just in the urine. It was transient too.

Vogt: I think he did both. However these of course are adrenal ectomized animals.

Long: Dr. Bush, has this compound any C_{11} activity so far as glycogen deposition is concerned?

Bush: That has not been tested.

Sayers: If it is a steroid have you thought about explaining its relatively high degree of polarity? Presumably it does not have a hydroxyl group at position 17?

Bush: We do not know that.

White: Wasn't the infrared spectrum done?

Bush: The amount available was only one quarter of that needed for the infrared using the best microcell available. Work is going on now and Professor Reichstein is trying to isolate much larger quantities in

mineralo-corticoid output has been the statement that although hypophysectomized animals and cases of Simmond's disease do not show the excessive salt loss that is shown by the Addisonian or the adrenalectomized animal nevertheless both sets of patients and animals show exactly the same deficiency as regards the excretion of a sudden water load or resistance to water intoxication as do adrenalectomized animals. It seems however that in hypophysectomized animals and in Simmond's disease salt retention is adequate even though one function which depends upon the adrenal the ability to respond adequately to a sudden change in water or electrolyte concentration of the body is definitely impaired. We class both phenomena under electrolyte and water metabolism but they are not necessarily influenced by the same type of adrenocortical hormone. It seems better therefore not to assume that this substance is the predominant one with regard to all the processes involved in electrolyte and water metabolism just because it makes up two thirds of the total mineralo corticoid activity in adrenal venous blood in one form of assay.

Thorn I say amen to that. It is very exciting that a substance of the salt retaining capacity of this substance has been demonstrated in the adrenal venous blood. However I do not think one can attempt to interpret the causes of salt retention under various conditions of adrenal activity for as I said earlier substances such as compound E and F under certain circumstances can be shown to have a profound sodium retaining capacity in intact man. Thus it seems possible that when the adrenal is stimulated excessively salt retention can be explained satisfactorily by the large output of substances such as E and F. However since in most instances sodium balance is maintained without any evidence of overactivity of the adrenal such as Cushing like syndrome we must conclude that substances more active in their salt retaining capacity than E or F must normally be secreted. When assaying biologically salt retaining factors obtained from extracts one must be very careful as quite different results can be obtained with the same material depending upon the route of administration and the interval and time of administration. Thus if compound E or F is given by mouth there may be minimal salt retention whereas the same quantity of hormone continuously infused intravenously may give profound salt retention. It would appear to me that the adrenal secretes multiple hormones many having salt retaining capacities of various degrees which may in part be modified by the state of organism.

Vogt May I ask one more chemical question? Is the assumption that one is dealing with a very small quantity of material which has large effects entirely based on the intensity of the triphenyltetrazolium reaction or is there other evidence? Is it possible that the triphenyltetrazolium evidence is misleading?

think are the 3 hydroxy analogues of compounds A B and S All those have been found not to be active

Long Is it not active on the carbohydrate and protein metabolism?

Bush No One problem arising from these results is that two thirds of the salt activity in this assay of adrenal venous blood in the dog was due to what chemically amounts to about two thirds of one per cent of the known active steroids detected In other words one has to talk about this material as if it were relatively specific as a mineralo corticoid The amounts of compounds F and B in the blood when converted into cortisone equivalents using the data of Pabst *et al* for the relative glycogenic activity and compared with Dr Vogt's figures (27) for the amounts of biologically active material in adrenal venous blood are quite sufficient to account for the biological activity found there In other words there is no necessity to postulate the existence of any other glucocorticoid in the adrenal blood in addition to those which are known chemical compounds

Thus it seems that this substance which is present in about two thirds of one per cent of the total chemically detectable material is responsible for two thirds of the total salt activity and little if any of the glucocorticoid activity However although the lack of glucocorticoid activity may make this compound specifically a mineralo corticoid one has to guard against saying that as regards the adrenal secretion it is the specific mineralo corticoid Apart from the fact that the steroids mainly responsible for the electrolyte balance of the extrarenal tissues may differ from those responsible for the electrolyte metabolism of the kidney tubule there is also the possibility that different types of electrolyte metabolism may be affected by different steroids Therefore although compounds E and B make up only one third of the so called mineralo corticoid activity of the adrenal blood on the basis of this assay in certain situations these compounds may be none the less more important than the so called mineralo corticoid This possibility is indicated by the experiments of Danford *et al* (28) which are extremely interesting because they could be taken as strong evidence for the unitarian hypothesis They showed that rats put on a low sodium diet for some time apart from showing a rise in 17 ketosteroid excretion also showed thymus atrophy of just the kind caused by the so called glucocorticoids or by injections of ACTH Thus whether or not the secretion that was required to meet the stress of salt restriction was a glucocorticoid there was a hypersecretion of glucocorticoids and they rather than the mineralo corticoid if the rat secretes such a substance may have been responsible for combating the stress

One of the arguments against the assumption of what Dr Sayers called the autonomous concept of adrenal regulation as regards

steroidogenesis. We suggested two paths of synthesis, one a 17 desoxy group and the other a 17 hydroxy group, progesterone being presumably the key precursor.

The dotted lines in Figure 19 indicate conversions of which we had no definite evidence on the basis of isolation of the indicated compounds, whereas the solid lines indicate definite isolation and identification. There are obviously a number of gaps in this diagram, but since it was drawn up, we have managed to test a few of them. The principal one is the question whether cholestenone is a possible precursor of any of these steroids.

When radiocholestenone, which was labeled at C₄ in ring A, was perfused without ACTH and with ACTH through a battery of beef glands, the counts on compound F and compound B, which are always the principal corticosteroids found in these perfusates, were zero. There was the usual increase in total output of F and B after ACTH*. This suggests that, at least under the conditions of perfusion that we employ, the F and B cannot originate from cholestenone. So that part of the diagram can be more or less discarded.

Drs. Stone and Hechter have reinvestigated the cholesterol conversion about which Dr. Hechter spoke at the last meeting. You will recall that we were very much interested in where ACTH exerted its activity in stimulating synthesis from cholesterol. Although we stated our belief that the action of ACTH must be involved in the degradation of cholesterol to some pregnenolone or progesterone-like substance, this was pure speculation. We have some data now which makes it somewhat less speculative. Data on what happened when radiocholesterol was perfused without ACTH and with ACTH. Here again, in terms of absolute weight of the principal compounds F and B, there was the increase that would be expected. Also, the specific activity increased in the presence of ACTH. Without ACTH, a total of 298 counts per milligram per minute was observed in F plus B, whereas in the presence of ACTH, with the same amount of cholesterol, 7900 odd counts were obtained, a very large increase indeed, roughly an increase of 25 times in the total radioactivity.

In another experiment, which I think is useful in the study of the scheme of corticosteroidogenesis, Drs. Stone and Hechter perfused progesterone labeled at C₄ through isolated bovine adrenals, without and with ACTH. The amount of radioactive F and B obtained without ACTH was 0.31 mg, and with ACTH 0.89 mg, but the total radioactivity increased from 2.12×10^3 to 2.42×10^3 , or less than 15 per

*Stone, D., and Hechter, O.: Unpublished data.

cent Moreover 5 ketolic companion substances observed in the chromatograms which increased from 0.90 before to 1.44 mg after ACTH had corresponding counts of 10.6×10^3 and 12.8×10^3

Keeping in mind the fact that we elute these from papers that may introduce materials which affect the count the over all picture is fairly clear that is the enormous increase observed when cholesterol was used as the precursor was not evident when progesterone was used as a precursor in the presence of ACTH This suggests then that ACTH does not act particularly in the conversion of progesterone to the products thereof in a progesterone perfusion

Another interesting feature of these data is that every single one of the five unidentified ketols is radioactive after the administration of radioactive progesterone which again points to progesterone as the probable precursor of the array of ketols that we have previously discussed Thus we come back again to our original supposition that the action of ACTH is somewhere back of progesterone presumably concerned with the degradation of cholesterol

For those of you who want more classical evidence about the nature of the products Figure 20 represents actual isolations and identification by infrared and ultraviolet of compounds appearing in a single experiment in which 550 mg of progesterone was perfused through four adrenal glands for $6\frac{1}{2}$ cycles in a little over three hours from an aliquot equivalent to 500 milligrams Dr Levy and his colleagues have isolated all of these compounds from a single perfusion This is quite a feat

It will be noted that the ratio of F to B is not necessarily one to one In Figure 20 the ratio is something like 4 to 1 or 3 to 1 There is more of F In addition 11 hydroxyprogesterone has been obtained A reduction product of 17 hydroxyprogesterone has been obtained 17 hydroxyprogesterone itself and other reduction products including the 3 ketone as well as the 3 hydroxy derivatives The configuration at C_5 is always the allo configuration We have never yet seen any compounds with the normal configuration at C_5 in any of our preparations

White Have you done this with fewer cycles with the thought that you might perhaps get a clue as to the sequential order in which the various compounds may be formed?

Pincus With radioactive progesterone we have some single-cycle data

White What do you find?

Pincus Unfortunately we are now depending upon paper analysis and until we really get that straightened out I can't give you the picture The thing that always surprises us is whether we use 6 cycles or as in one of the previous experiments 3 cycles compounds F and B predominate The other compounds tend on the whole to be present

ISOLATION Aliquot equivalent to 500 mg

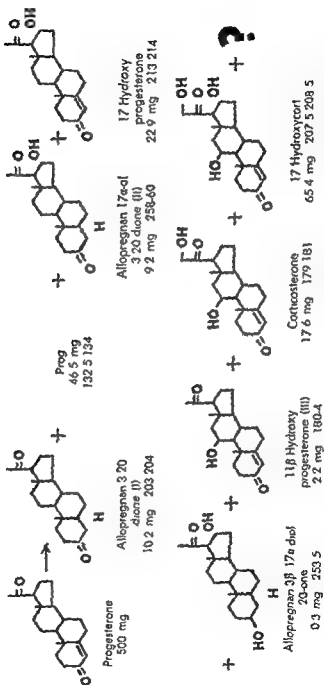


FIGURE 20 Perfusion of progesterone 550 mg 8 liters of whole blood various flow throughs 5 lacerated adrenals $5\frac{1}{2}$ cycles in 3 1/6 hours. All compounds except I II and III have been isolated from adrenals. Based on unpublished data of Levy Jeanloz Marshall and Jacobson

rats sheep and cats there is a relatively small amount of a Δ^4 3 keto-steroid which is very similar to 11β hydroxyandrost-4 ene-3, 17 dione but which at present seems more likely to be 17 hydroxyprogesterone. It is found only in very small amounts and the identification is by no means complete.

In three cases of adrenal tumors which I was lucky enough to have a chance of studying with Dr L. R. Broster who was operating on them we took adrenal venous blood from the left adrenal vein during the operation for removal of the tumors and we found apparently the same substance in each case. The amounts were very small in two of the cases suffering from Cushing's syndrome with little virilism. In the third however a case of long standing virilism with no signs of Cushing's syndrome large amounts of this substance were found and the amounts of 17 hydroxycorticosterone were extremely small the reverse of the picture in the other two cases. Dr J. Jailer* has speculated that 17 hydroxyprogesterone may be the androgen or the androgenic precursor in adrenal virilism on the basis of metabolic studies which he has done and because of the fact that on the basis of the Worcester group's metabolic scheme for the synthesis of corticosteroids in the adrenal it could be supposed that adrenal virilism was some form of inborn metabolic disease of a comparatively simple kind causing the course of steroid synthesis to stop at that stage. It would explain the production of this compound the tendency of these patients to have Addisonian symptoms and also the overstimulation of these glands. But there is some difficulty over this theory at the moment because although I think Dr Wintersteiner first reported that 17 hydroxyprogesterone was androgenic there is now doubt about this. I gather that you Dr White have found that it is active by oral administration in the rat is that right and not by the comb test or am I misquoting you?

W. Hite: Not entirely. It is a good example of how impressions become information though!

Bush: I can show you a letter if you want me to.

W. Hite: Oh no not at all. I shall present what information I have. Only recently has 17 hydroxyprogesterone become available in large quantities and there are several biological studies in progress including an examination of its possible androgenic and progestational activity. Of the data which are completed Dr Gassner has studied the effect of 17 hydroxyprogesterone by injection on the chick comb. The steroid was weakly active as an androgen. The compound was also assayed in chicks by the oral route at a dose of 15 mg per kg of food comb weight was increased about 50 per cent over the controls. Androstene

*Personal communication

in smaller amounts. The only substance other than F which is rather large in quantity is the 17 hydroxyprogesterone. The amount given in Figure 20 is the largest amount of 17 hydroxyprogesterone in percent age yield that we have ever obtained in any experiment. In previous experiments, we obtained only a few milligrams for example in a 3 cycle experiment. Whether we can by single cycles get indications of a stepwise change is something in which we are very much interested for that we are going to depend on the radioprogestosterone studies. I think for the isolation studies in order to get an adequate amount of any product we have to recycle at least a few times.

That is about all there is to add at present to what we have reported over the past few years. I think that slowly but surely we shall be able to identify all of the compounds coming after ACTH. In my opinion the most interesting thing we can do is to see what kind of check we can find between those compounds and what happens after progesterone is perfused. So far of the 15 regularly obtained, we are certain of only 7. We still have 8 more to worry about.

About the biological activity every ACTH perfusate that we have used has sodium retaining activity. We don't know where the sodium retaining activity lies because we haven't come to the point yet of analyzing for the sodium retaining factor.

White: I should like to make a few comments that relate to 17 hydroxyprogesterone and also to the question of what the urinary keto steroids really represent in terms of androgen production by the adrenal. Dr. Joseph Jailer at Columbia has had the opportunity of studying a number of cases of adrenal virilism and as you know if cortisone is administered to these individuals there is a marked suppression of the output of ketosteroids. Dr. Jailer thought that it might be possible that 17 hydroxyprogesterone contributes to urinary ketosteroids and in this connection I was interested that so relatively large an amount of 17 hydroxyprogesterone turned up in Dr. Pincus' perfusion studies.

Dr. Jailer obtained a base line for excretion of 17 ketosteroids in his studies of adrenal virilism. He then administered cortisone and obtained the expected decline in 17 ketosteroid excretion. While continuing the cortisone administration 17 hydroxyprogesterone was also given. Under these circumstances there was a marked rise in the 17 ketosteroid excretion. If one can make the long jump it suggests that a C₁ corticoid can contribute to the 17 ketosteroid excretion. Dr. Bush: I wonder if you would be good enough to tell us about the observations which you talked about at the Laurentian Hormone Conference a few months

ago

Bush: The observations are these. In the adrenal venous blood of

his colleagues carried out similar experiments in rats and have recently reported their results which confirm ours in all details

Sayers The possibility exists that the cortical steroids are degraded to 17 ketosteroids in the organism. However I think we should seriously consider some of the degradation as being due to manipulations of the chemist after the urine is collected. Mr Glenn and Dr Nelson of the University of Utah have shown that if urine is handled very gently with glucuronidase hydrolysis and then subjected to chromatographic separation an increase in 17 ketosteroids after the administration of ACTH is not obtained. They had one patient a castrated male who excreted no 17 ketosteroids by their technique but did excrete C 21 17 hydroxycorticoids. And they had patients with Addison's disease who secreted 17 ketosteroids but no C 21 17 hydroxysteroids.

Thorn Were the Addisonian patients males or females?

Sayers These were male Addisonians. If urine is treated by the routine acid hydrolysis technique then an increase in 17 ketosteroids does follow ACTH administration. Part of this increase may be due to conversion of C 21 steroids to substances which give a color in the Zimmerman reaction.

Sayers This does not however explain the difference between oral and parenteral administration. I should like to hear some other comments about liver dysfunction and 17 ketosteroid excretion.

Lukens I should like to include this question. As I understand it ACTH causes a marked increase in urinary corticoids of the E type in patients with Cushing's syndrome. Does ACTH cause a disproportionately large amount of 17 ketosteroids when given to a patient with virilism?

Bush I think the answer is yes. That was done by Albright and Wilkins.

Sayers Dr Bush shouldn't that be reinvestigated with these newer techniques?

Bush Do I understand that both methods have been done on the same urines of patients treated with ACTH?

Sayers No. Mr Glenn took normal urine and divided it into two parts. To one he added compound F and acid hydrolyzed both samples. An apparent increase in 17 ketosteroids occurred after the addition of compound F. This increase was due to background color development and disappeared when the Allen correction was applied. In most hospital laboratories this correction is not applied and at least part of the ketosteroid color is contributed by acid breakdown products of cortical steroids.

Bush That is interesting because it is a difficult question to settle due to the different types of conjugates split by acid and enzymic hydro-

dione 3,17 at this dose level increases comb weight by 340 per cent. We have no data in rats at this time.

The statement is made in the literature (29) that this compound has been shown to be an androgen and lacks progestational activity. Earlier in our discussions Dr. Pincus said, I believe, that after a severe trauma such as burn there is an initial very marked rise in 17 ketosteroids followed later by an increase in corticoids. One might ask whether in view of all that has been said 17 hydroxyprogesterone is not important in the genesis of adrenal cortical steroids. With a sudden activation of the hypophysial-adrenal cortical mechanism certain of the adrenal cortical steroid intermediates might escape from the gland. Since Dr. Jailer's work indicates that 17 hydroxyprogesterone may contribute to 17 ketosteroid production the data again emphasize the caution which should be exercised in interpreting 17 ketosteroid production as evidence of androgen secretion.

Conn: I should like to agree with what Dr. White has just said and to recall some of the data presented at this Conference last year in this connection. We had found that if one gives free compound F to normal people by mouth there is a very large excretion of 17 ketosteroids in the urine whereas if the same amount of free compound F is given intramuscularly this rise in 17 ketosteroid excretion does not occur. The overall metabolic effect of compound F by the two routes was comparable. We then went to compound S which we found to be completely inactive from a metabolic point of view. But when we gave free compound S by mouth there was a four to five fold increase in 17 ketosteroid excretion whereas when free S was given intramuscularly the rise of 17 ketosteroid excretion failed to occur. On the basis of these findings we made the assumption that when these precursors of 17 ketosteroids which are not androgenic come to the liver in high concentration via the portal system the conversion to 17 ketosteroids is likely to be intense and further that this conversion appears to be a hepatic function.

Therefore we went on with this type of study in patients with hepatic disease and we found that in patients with chronic liver disease this conversion was greatly impaired. When we gave large amounts of compound S or compound F by mouth to patients with hepatic disease we obtained very small increases in 17 ketosteroid excretion as compared to the normal individual. More recently we have done multiple studies over a long period of time on a patient gradually recovering from an intense attack of acute hepatitis. We obtained the same results in the initial stage that we had observed in the chronic hepatitis cases. But as progressive recovery ensued the ability to make this conversion to 17 ketosteroids gradually returned. Following our report Corcoran and

ketoetiocholanolone arise from both E and F. But from adrenosterone 11 β hydroxyandrosterone and 11 ketoandrosterone are obtained as well as the 11-oxygenated steroids in the etiocholane series.

Kendall Is the oxygen present as a ketone or a hydroxyl group?

Pincus From E and F there is more of the 11 β hydroxy 17 keto steroids but lesser amounts of the 11 keto derivatives have also been isolated. This suggests that the 11 oxygenated metabolites in the androstane series are only minor derivatives of E and F and points to a compound like adrenosterone as the major source. The point is that from E and F one does not get such 11 desoxy ketosteroids as androsterone and etiocholanolone. In other words it looks as though the capacity to remove the 11 oxygen function is very limited in the human. If it occurs at all the amounts are so small that they cannot be isolated. Dr. Thorn has given heroic doses 500 mg a day of the substance to patients and yet no measurable amounts of 11 desoxy ketosteroids have been found. So what is the precursor of etiocholanolone? Etiocholanolone as well as androsterone arise from Δ^4 androstene 3,17 dione which has been isolated from adrenal tissue.

In the case of Dr. Jailer's experiments unless he can identify the specific substance you see the difficulty that one gets into in terms of the true 17 ketosteroid compounds in human urine. Because in human urine there are roughly equal amounts of androsterone and etiocholanolone but lesser amounts of the 11 oxygenated C_{19} steroids the available evidence would suggest very strongly that the urinary 17 ketosteroids don't have a single precursor but maybe five or more.

Thorn That brings up another point which has not yet been discussed that is the varying degree of biological androgenicity of the 17 ketosteroids as they are derived from these specific substances. These analyses are time consuming and technically difficult to carry out. We have had the help of Dr. Paul Munson at the Harvard Dental School and have been able to make some progress in this direction. One must select arbitrarily a biological assay for androgenicity. I was wondering during the discussion of 17 hydroxyprogesterone whether such activity may show up in the comb test and how these assays correlate with what happens when the material is given to a normal female patient. We can state on the basis of our experiments with Dr. Munson that compound F gives rise to 17 ketosteroids and that these 17 ketosteroids have good androgenic potency as measured by the comb test. Furthermore the quantity of androgen biologically assayed derived from compound F is much greater than that from an equivalent quantity of corticosterone.

White I seem to have missed something. I am not quite clear as to how these data are in harmony with the information given by Dr. Pincus.

lysis When he added compound F to the urine did he hydrolyze both urines with acid?

Sajers Yes

Bush How was the 17 ketosteroid determination done and was an absorption curve done?

Sajers Yes When the Allen correction \square applied added F does not result in an appreciable increase in the 17 ketosteroid fraction

Bush That is quite interesting because Dr Lieberman* has recently boiled compound P with hydrochloric acid under the conditions for the urinary 17 ketosteroid determination and found no production of 17 ketosteroids at all They have done it at Utah with compound F and found that although a certain amount of dirty color \square shown with m dinitrobenzene on testing the absorption curve it is not that of a 17 ketosteroid

Sajers Incidentally the paper of Holtorff and Koch published in 1940 (30) pointed out that the Zimmerman reaction is not specific for 17 ketosteroids Substances such as compound A give a color reaction which corresponds to about 20 per cent of the value that would be expected of a 17 ketosteroid

Pincus We have some information on this First of all after glucuronidase hydrolysis 17 ketosteroid \square obtained

Thorn Do you mean an increase with ACTH?

Pincus No in the urine of normal persons That 17 ketosteroid is quantitatively not comparable to the amount obtained from the aliquot of the same urine which has been acid hydrolyzed

Sajers Dr Pincus did you perform a chromatographic separation of the 17 ketosteroids and the cortical steroids?

Pincus Yes these were all separated

The second point that I should like to make about Glenn's work is that he should identify the substances He should separate them Dr Dorfman and Dr Savard in our laboratory have been studying with Dr Thorn's help the metabolism of compounds F E adrenosterone and S and examining the 17 ketosteroids which arise in the urine following the administration of these materials This is on the basis of isolation work not by any other method In other words the compounds are isolated

After the administration of E and F but not after S 11 oxygenated 17 ketosteroid \square found This of course is what would be expected and it is nothing unusual But the interesting feature of the data is that after F the 11 oxygenated 17 ketosteroid is practically all in the etiocholane series In other words 11 hydroxyetiocholanolone and 11

*Personal communication

ketoetiocholanolone arise from both E and F. But from adrenosterone 11 β hydroxyandrosterone and 11 ketoandrosterone are obtained as well as the 11-oxygenated steroids in the etiocholanone series.

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White I seem to have missed something. I am not quite clear as to how these data are in harmony with the information given by Dr. Pincus.

Pincus All of the substances in the etiocholane series appear to be inactive as androgens. All of the substances in the androsterone series are active.

White When you say active was Dr. Munson's bioassay in chicks?

Thorn That is correct.

White This is interesting because a compound like androstenedione 3-17 while active in bioassay in chicks is distinctly less active in the rat. Again one has to define what one means by androgenicity.

Pincus In terms of bioassay, Dr. Munson's data would suggest that there may be substances that synergize with the trace amounts of androgen present.

White Androstenedione is without known androgenicity in man in terms of trying to establish it as an adrenal virilizing androgen. I am trying to limit the possibilities.

Bush This substance which is so similar to 17-hydroxyprogesterone occurs in the urine of normal untreated females in about six times the amount found in the urine of normal untreated males.

White Dr. Bush, as far as adrenosterone versus 17-hydroxyprogesterone, could not one distinguish between these two?

Bush Yes, but so far this has not been done.

White Can you differentiate by a color reaction, i.e. for 17-keto steroids?

Bush No, not in these tests.

Pincus You haven't tried the Zimmerman reaction?

Bush No. We have had only very small amounts of 17-hydroxyprogesterone and 17-hydroxyprogesterone gives an indistinguishable color on paper. I find I think Dr. U. Savard finds a different color.

Pincus Have you had any indication in these venous effluents of C_{19} compounds?

Bush Not surely, no, but in one of the washing procedures I think anything less polar than desoxycorticosterone would suffer quite considerable loss. I have usually done a Butenandt separation which of course would lose androstenedione.

Pincus Nelson and Samuels originally thought that they had 11-hydroxyandrostenedione or something like that in the blood from the dog and we are just starting a project for the isolation of C_{19} compounds from adrenal perfusate. There is no doubt in our minds that there are some there. The only question is as to their chemical nature. We are still very much in the dark.

Thorn To revert back to the studies in regard to liver function we have been interested for some time in the effect of disorders of liver function on steroid metabolism. Our technique has been to administer approximately 500 mg. per day of a steroid to a patient with acute

hepatic disease. We have usually given 1.5 to 2.5 Gm total of steroid. Later when the patient has recovered we repeat the same dose and study the difference in the type and quantity of urinary steroids excreted under these two circumstances. These studies we hope will assist us in our interpretation of changes in urinary steroids when liver function is impaired.

We have had one interesting experience. I hesitate to report a single case except that it seems to be so well documented. Ordinarily 50 mg of cortisone given by mouth will induce a significant eosinopenia. In a particular patient with severe hepatitis 50 mg of cortisone by mouth or intravenously did not produce an eosinopenia whereas 50 mg of compound F did. Following recovery from acute hepatitis this patient showed a marked eosinopenia when 50 mg of cortisone was administered. It would appear in this instance that cortisone was relatively ineffective in producing an eosinopenia when administered to this patient in the presence of acute liver disease whereas ACTH and compound F gave the expected eosinopenic response both during the phase of acute hepatitis and later in recovery.

Pincus That is what we were talking about the last time that cortisone probably is transformed to F in order to be effective or to some other substance.

Long Does that occur in a joint cavity too?

Pincus We have injected both cortisone and F into a joint and tried to recover the products. Cortisone is not transformed to F in the joint.

Long Does it affect the disease process of the joint?

Pincus It doesn't affect the disease process in the joint.

Long But F does?

Pincus Yes.

White Cortisone does work in the eye doesn't it?

Lukens Yes the apparent difference between the local effect of cortisone in the joint and in the eye has not been explained.

Pincus I have the impression from what I have heard from people working in this field that if cortisone is given in a very large dose into the inflamed joint there may be an effect but the dosage of F that is necessary is certainly much smaller.

Astwood Yes but it is not an all or none phenomenon.

Pincus We have not been able to find any significant amount of F in the few inflamed joints from which we have withdrawn fluid.

Long Isn't this a particularly important point to emphasize that possibly the effects of cortisone are dependent upon a preliminary transformation into F or maybe some other compound?

Lukens In studies which are in progress in Dr. Hollander's clinic the actual conversion and location of these compounds is being meas-

ured It looks as if the compounds were located in the tissues in different ways I am sorry I can't give the details but the extent to which they are attached to or absorbed by the cells of the joint membrane differs for these two compounds

Long I think this very important when one thinks of all of the attempts that have been made to demonstrate an effect of cortisone on isolated liver slices and other tissues Maybe a conversion to compound I, or something else is required in order to get the metabolic effect

Selye On the basis of our experiments with the pneumodermal test hydrocortisone injected directly into ordinary skin (outside the pneumodermal region) does have a definite though extremely slight effect upon the surrounding cutaneous structures This action is enormously enhanced if the skin is detached by the pneumodermal technique Presumably, local conditioning of the hydrocortisone effect by the dystrophy of this type of skin is responsible for the cutaneous atrophy thus induced The same is true of cortisone under these conditions although its effects are less pronounced and less long lasting I think that these observations plus the local effects of cortisone treatment in ophthalmology and injection of hydrocortisone into joints with limited local effects definitely prove that both cortisone and hydrocortisone have topical actions as such without having to go through the liver or other parts of the body in order to be activated

Conn Baker and Ingle have shown that same local effect of cortisone

Selye Yes I mentioned their experiments on the application of alcoholic solutions to the skin in the course of my presentation

Pincus Your work is in the rat There may be a species difference

Thorn When cortisone and hydrocortisone are given by mouth in equivalent quantities the 17 ketosteroids and the biologically active androgens derived from the two compounds are quite different

Selye I wonder to just what extent increased fixation in the tissues is an important factor there The most striking thing in our experiments with the pneumodermal technique was that hydrocortisone stayed so long at the place where it was injected because of its slow absorption rate No other steroid that we have tried so far stays in the pneumodermal wall as long as hydrocortisone

Thorn That was the acetate?

Selye Yes we gave it as the acetate The cortisone was also given as an acetate in this experiment

Thorn The metabolic effect of a given amount of cortisone acetate injected is quite different from that of hydrocortisone acetate

Selye I should like to know how we can be sure that this is not due just to better fixation or slower absorption of the compound without assuming any qualitative difference?

Thorn We assume that it is due to those things because the free compound is much more active when injected than the hydrocortisone acetate

Pincus I should like to point out that in the assay that Dr Dougherty described we have been comparing E F B and a number of other compounds and to our very great surprise although we should have known it from Dr Dougherty's original remarks E has about one one hundredth the activity of F in that test whereas corticosterone B is if any thing only slightly less active than F

Thorn Which test is employed in this instance?

Pincus That is the anti inflammatory or antiphlogistic test in which Dougherty injects into the same site histamine plus the corticosteroid We have quantitated that test for assay and the possibility is again suggested that the 11 hydroxy function is very important for the particular effect

White From Dougherty's data if one takes compound E as 100 then compound F is about 7 000 i.e. 70 times as active as E but compound B is 5 or 6 i.e. approximately one twentieth as active as E

Thorn If you were using compound F as your standard you would state that corticosterone and cortisone were very much alike wouldn't you?

White Compound B is less active than cortisone There is one other local effect that I think is of interest Dougherty finds that whereas compound F causes lymphocytolysis *in vitro* cortisone does not

Pincus What does compound B do?

White I do not know whether compound B has been tried for lymphocytolysis *in vitro* Compound B has limited effect *in vivo* in so far as lymphoid tissue changes are concerned

Pincus In answer to Dr White's question if there is any basis for the difference between 11 hydroxy and 11 keto as between E and F it is certainly much more specific than the general polarity of the compound that is involved

Long It certainly is

Bush It must be a more specific effect than simply the difference in solubility

Astwood Dr Bush if compound F is more polar than compound E why is it more slowly absorbed into the aqueous body fluids?

Bush I do not know

Pincus Polarity in this particular system isn't necessarily proportionate to water solubility The systems that are ordinarily used are organic solvents propylene glycol toluol or something of that sort

Pincus We have tried combinations in the Dougherty test There doesn't appear to be any sign of antagonism That doesn't mean how

ever to exclude the greater affinity. It merely means that the affinity may be so great that it just can't be overcome with the doses of cortisone used.

Bush Quite a strong indication that these differences in effect may not be simply related to polarity is the recent work by Dr. Schellman, at Utah in which he has studied the association of steroids with serum albumin. It has been known for a long time that testosterone is comparatively insoluble in water but extremely soluble in serum albumin solution. What he has found is that this association with steroids is dependent on short range van der Waals forces as much as on interaction with polar groups. Thus any simple rules based on their water solubility or their apparent partition coefficients in chromatographic systems may be extremely untrustworthy.

Loew Are very special conditions needed for the lymphocytolysis shown by Dougherty?

White Not particularly. The steroid is added to buffy coat of blood a blood smear made within 30 seconds and the number of altered lymphocytes counted after staining of the smear.

Loew Is this published?

White He spoke about it here at the Macy Conference (31) several years ago and has a paper in press. We tried for a number of years to demonstrate it with adrenocortical extract quite unsatisfactorily. But he recently has been able to do this with compound F.

White I don't know to what extent this has been confirmed. There has been controversy over whether this happens *in vitro*. Dr. Schrek and Dr. Feldman have observed these changes. Dr. Robertson in England was unable to demonstrate it. Dr. Ingle have you had any experience with this?

Ingle No.

Pincus Dr. Hechter several years ago tried several steroids but was unable to get a direct effect of any.

Loew I think Hechter got it when he added a part of lymphoid glands to the serum.

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ADRENALECTOMY IN MAN*

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ADRENALECTOMY IN MAN is done either for therapeutic or investigative reasons. There are three principal conditions in which it has been tried: first, carcinoma of the prostate or breast for which it may be regarded as palliative treatment. The relief of pain and the modest degree of retardation of tumor growth are significant in some patients, although the operation is in no sense a cure for cancer. The second important indication for adrenalectomy in man is Cushing's syndrome, about which Drs. Sprague and Conn have previously reported at these meetings. Here I think that adrenalectomy should be regarded as curative, certainly if the best results obtained in this condition can be confirmed and repeated. Third, adrenalectomy has been tried in a number of clinics for severe hypertension. In this instance the procedure is distinctly investigative. It is in investigations on this aspect of adrenalectomy that I have been associated with Drs. Wolferth, Zintel, Hafkenschiel, Hills, and others (1, 2). A series of 69 patients have been subjected to adrenal surgery up to September 1, 1952, which is the date I have used for preparing these data.

The indications for adrenalectomy are listed in Table III. The patients are severe hypertensives, as shown by the high diastolic pressure. These are patients who have not responded to intensive medical care, which now includes the use of hexamethonium, apresoline, and other drugs for hypertension. They are usually patients who are not suitable for sympathectomy or ones who have failed to respond to sympathectomy. We demand adequate renal function, as shown by the blood urea nitrogen (BUN) and phenolsulfonphthalein (PSP) excretion. We were not as rigorous about this in the early days of the study, and in fact we deliberately performed adrenalectomies on two individuals with renal

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insufficiency in the hope that it might give these very ill patients some improvement. But now we insist on adequate renal function.

Thorn Would you wish to qualify that statement? Is that on an unrestricted protein intake or not?

Lukens That is on an unrestricted diet.

The last item in Table III progressive vascular damage simply means that the damage of *angina congestive failure retinitis* and so forth is adverse to the prognosis of the patient.

TABLE III
Indications for Adrenalectomy

- | | |
|---|---------------------------------------|
| 1 | Diastolic BP 120 mm Hg or more |
| 2 | Under 35 years old |
| 3 | No response to intensive medical care |
| 4 | BUN less than 20 mg per 100 ml |
| 5 | PSP excretion at least 15% in 15 mins |
| 6 | Progressive vascular damage |

As listed in Table IV 69 patients have had extensive adrenal resection. Adrenalectomy has sometimes been done alone but in the majority of patients it has been combined with various forms of sympathectomy usually of the Adson type. I shall not discuss the role of sympathectomy although it will have to be evaluated in the final analysis. For the present it may be noted that these patients have all had adrenalectomy followed in most instances by adrenal insufficiency. 58 of the patients requiring substitution therapy. After adrenal surgery 11 of the 69 patients needed no substitution therapy. This means that 58 patients have had enough adrenal tissue removed to determine the effect of

TABLE IV
Data to September 1 1952

	No	Per Cent
A Patients with extensive adrenal resection (subtotal or total resection with or without various forms of sympathectomy)	69	100
Total mortality to date	17	25
B Of the first 34 cases		
1 No surviving 15 to 29 months	22	
2 Died in first year	9	35
3 Died after first year	3	

adrenal insufficiency on the hypertension. Of 22 patients living (September 1 1952), 9 need no replacement i.e., 13 are available for the study of the more prolonged effects of adequate adrenalectomy.

The replacement therapy of these patients has been essentially that described at these Conferences by Thorn Sprague Conn and others (3) for Addison's disease. At the time of removal of the second adrenal the pre and post operative treatment currently consists of 50 mg of cortisone the morning of operation (a little less than we used to give) and 100 mg of cortisone a day for one or two days after operation. The dose of cortisone is then tapered to 50, 37 and 25 mg of cortisone per day within seven to ten days if all has gone well. If total adrenalectomy is done 1 to 2 mg. of desoxycorticosterone are added beginning the day after operation. Generally speaking in these patients we have tried to use as little desoxycorticosterone as possible because of experimental studies showing that DCA is a hypertensive agent.

For the final maintenance therapy of these patients 25 mg a day of cortisone and 1 to 2 mg of desoxycorticosterone may be used. There is a range of dosage. For example as little as 4 mg of cortisone has been sufficient in some patients and as much as 50 mg has been used for short periods of maintenance in others. In connection with immediate postoperative care there is another point. These patients have required let us say 100 mg of cortisone a day but Drs Sprague Conn and Shorr (4) have used 200 to 300 mg a day in Cushing's syndrome in connection with adrenalectomy. Thus it appears that the cortisone requirement of patients with different diseases may differ considerably.

Thorn: We can confirm this statement but wouldn't you prefer to qualify it and say that it is not necessarily so much a difference in diseases as the fact that the patient with Cushing's syndrome has been adjusted for years to a very high level of hormone.

Lukens: That is better worded. Thank you.

Sayers: Have you carried out adrenalectomies on patients with Cushing's syndrome and actually demonstrated this difference in requirements?

Lukens: We have not done total adrenalectomy in patients with Cushing's syndrome in the last few years.

Selye: In addition to Cushing's syndrome can you mention any other disease in which the requirements are much greater?

Lukens: I suspect that they would be somewhat greater in removing an adrenal tumor for virilism in spite of the lack of systemic disease. I say that because in a few patients who have had tumors removed for adrenocortical virilism the severe postoperative febrile response that has occurred suggests the need for massive replacement therapy. By and

large good replacement therapy with cortisone abolishes these severe and undesirable responses to adrenal surgery. Would you agree with that Dr. Thorn and Dr. Conn?

Thorn I can only say that we have given large doses of hormone to these patients with good results.

Conn I would say exactly the same thing as Dr. Thorn. I don't think anyone has done careful studies to indicate whether or not the Cushing patient requires a much larger amount than a hypertensive in the post-operative period.

Lukens You have given the larger amounts as a precaution and they have worked well?

Thorn We have observed a poor clinical response in some patients with as much as 100 mg. of cortisone per day which from all of our experience with complete bilateral adrenalectomy in man in conditions other than Cushing's disease should be adequate. It would therefore appear that the patients with Cushing's disease may require much higher levels of hormone following operation and that a reduction in dosage must be carried out much more carefully and much more slowly. I have not had enough experience in regard to adrenal virilism independent of Cushing's syndrome to answer that point.

Lukens There are one or two other things. We have not noticed the undue sensitivity of these patients to the cerebral effects of cortisone which was described by Drs. Sprague and Conn at the last Conference (3). The patients appear to tolerate 25 mg. a day of cortisone with no unusual cerebral effect. That may be just a coincidence. A few of the patients have complained of stiffness of the joints when their therapy was reduced. It is not known whether these are pre-arthritis in whom the condition is revealed or just what it means. This stiffness is controlled by Addisonian dose levels of cortisone.

Pincus May I ask if you have given any replacement therapy with hydrocortisone?

Lukens Not in this series.

Sayers Dr. Lukens, I am interested in details. You reduce the dose of cortisone and keep the dose of desoxycorticosterone acetate fixed?

Lukens At times we stop all therapy in a patient for a test period. If subtotal adrenalectomy has been done and if the blood pressure is still high as it frequently is, substitution therapy is reduced to a minimum. In the course of this reduction these patients may develop early symptoms of adrenal insufficiency and it is in that connection that the stiffness has appeared. Desoxycorticosterone may or may not have been given.

Selye You did not try varying cortisone and desoxycorticosterone and following their effects upon the joint lesions?

Lukens No Stiffness has been a minor symptom We know of no objective findings which could be followed

All patients have had an excellent appetite on these small doses of cortisone so much so that obesity has become a problem in some of them This is obesity without edema as far as we know Since obesity is undesirable in patients with cardiovascular disease we have tried to prevent it

TABLE V

Classification of Response to Adrenalectomy

- | | |
|---|----------------------------------------------------------------------------------------------------------|
| A | Fall of blood pressure to normal (± 10 mm Hg) lying and standing Top normal blood pressure = 140/90 |
| B | Elevated blood pressure lying but postural fall to near normal |
| C | No change in blood pressure but improved symptoms or signs |
| D | No change in blood pressure and no improvement in symptoms and signs |

The response to adrenal surgery has been classified in our clinic for our own purposes as Table V shows In group A there is a fall of blood pressure to normal levels whether the patient is recumbent or standing and there is marked improvement in most of their symptoms The B and C groups have been benefited less but these patients are significantly improved as far as their symptomatology and capacity to live and work are concerned In that connection I would note our emphasis on the value of a postural fall of blood pressure There are quite a few patients with a high resting blood pressure who are very much relieved and whose cardiac size has reverted to normal apparently because of this postural fall It seems to reduce their cardiac burden The last group frankly recognizes the fact that there are many of these patients in whom no benefit can be obtained

Gellhorn What does postural mean?

Lukens The blood pressure falls on standing

Gellhorn Are these patients also sympathectomized?

Lukens Sympathectomy promotes postural hypotension but it will occur in patients with adrenalectomy without sympathectomy

Ingle Is the postural hypotension affected by desoxycorticosterone? I recall Dr Huggins saying that an occasional adrenalectomized patient treated with cortisone alone would develop postural hypotension which could be corrected by desoxycorticosterone

Lukens I believe that is true although we have not studied it In our effort to use as little replacement therapy as possible these patients

have been treated by the use of elastic stockings and girdles which relieve the symptoms of hypotension. We have used these rather than hormones.

Conn It has been our experience that postural hypotension in this instance as well as in Addison's disease is evidence of inadequate replacement therapy. All patients who have a postural fall of blood pressure and are taking small amounts of steroids get into trouble when exposed to stresses as mild as the common cold. When replacement therapy is raised to the point of postural hypotension being abolished the patient does not get into trouble with mild stresses but this dose may be sufficient to restore hypertension.

Lukens Our patients have been maintained for a life outside the hospital; they withstand the ordinary respiratory infections and so forth but they still have a modest degree of postural hypotension. We are probably keeping them at minimal essential maintenance which is about the goal.

In group D Table V there is no change in blood pressure and no improvement in symptoms and signs and this must be placed with category A in one's thinking. When these two groups are associated it places a problem clearly before us. There is a good response in some patients and no response in others.

In talking about these responses I have so far spoken as if all hypertensives were the same which of course is not true. We have used the Smithwick grouping of the severity of hypertension in order that our results might be comparable to those of other investigators. As Table VI shows we have not operated on any Smithwick group I patients.

TABLE VI

Smithwick group	Response to adrenalectomy				Total per group	Mortality (patients)
	A	B	C	D		
1	0	0	0	0	0	0
2	5	8	8	0	21	0
3	6	4	3	0	13	1
4	4	8	12	11	35	16

They are not ill enough and the prognosis of hypertension is very good in the early stages (5). The favorable effects of surgery in groups 2 and 3 are apparent. In Smithwick group 4 although the best type of response the A response can be obtained there is a significant mortality. In fact in this group it verges on 50 per cent. It will be less than this in the patients currently operated.

Long Would you tell us something about what the Smithwick groups 1 to 4 are?

Lukens The Smithwick classification of hypertensive cardiovascular disease is an effort to make a broad evaluation of the severity of the disease in its principal anatomical sites. The disease particularly involves the heart, the kidneys, the cerebral vessels, and the retinal vessels. The areas involved and the degree of the damage are tabulated in considerable detail and from the results a final classification is made. Smithwick group 1 is the mildest, group 4 the most severe stage of the disease. Omitting the detailed definition of certain terms, Table VII gives summaries of the evaluation of the clinical findings and Table VIII of the use of this data for the classification of the stage of hypertensive disease. The Tables have been based on a recent communication from Dr. R. H. Smithwick. Using this accepted procedure of classification, group 4 comprises the patients with the most severe arteriolar disease. Even though the mortality is negligible in our patients in groups 2 and 3, it would be a great help if we could select those hypertensive patients who would have an A type of response. This is a key problem, and I hope that this gathering will give me some suggestions. As Table IX shows, an A type of response has occurred in five patients who were on

TABLE VII
Factors Used in Grading the Cardiovascular Status of
Hypertensive Patients (Smithwick)

Factors	Numerical Value
Cerebrovascular accident with or without minor residual	1
Abnormal EKG	
Enlarged heart	
Impending cardiac failure	
PSP less than 25% in 15 minutes or less than 60% in 120 minutes	
Age 50 or over	
Mild angina	2
Cerebrovascular accident with residual symp- toms *	
Frank congestive failure moderate angina	
PSP less than 20% in 15 minutes	
Unsatisfactory response to sedation	3
PSP less than 15% in 15 minutes	
Nitrogen retention	4

Cerebral deterioration or hemiparesis

TABLE VIII

Classification of Hypertensive Patients According to the Criteria of Smithwick

Group	Numerical Grade	Other Factors
1	Less than 4	<p>Eye grounds grade 0 to 1</p> <p>No change in cerebral cardiac or renal areas</p>
2	Less than 4	<p>Eye grounds grade 0 to 1 with changes in cerebral cardiac and/or renal areas</p> <p>Eye grounds grade 2 3 or 4 with or without changes in cerebral cardiac or renal areas</p>
3	4 or more	<p>Resting diastolic level below 140 mm</p> <p>Changes are present in cerebral cardiac and/or renal areas but they do not include the following</p> <p>(a) Cerebrovascular accident with marked residual symptoms</p> <p>(b) Frank congestive failure</p> <p>(c) PSP below 15% in 15 minutes associated with a poor response to sedation</p>
4	4 or more	<p>Resting diastolic level below 140 mm combined with one or more of the following</p> <p>(a) Cerebrovascular accident with marked residual symptoms</p> <p>(b) Frank congestive failure</p> <p>(c) PSP below 15% in 15 minutes combined with a poor response to sedation or</p> <p>Resting diastolic level of 140 mm or more</p>

TABLE IX

Results of Adrenalectomy after 15-29 Months

Type of Response	15 Patients on Replacement Therapy	9 Patients Not on Replacement Therapy
A	5	1
B	3	2
C	5	6

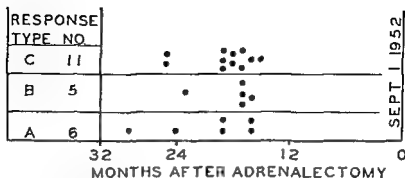


FIGURE 21: Twenty two patients followed for a year or more. Type of response (A B C) is defined in Table V

replacement therapy and in one patient who did not need replacement therapy but who nevertheless had one of the best responses. I can only say that she was an unusual patient. Figure 21 shows the situation in graphic form. This again focuses attention on the problem of the selection of these patients.

Pitts In those that have been followed the longest with an A status initially, has there been any tendency to shift to a B?

Lukens Many of those who have had an A type of response within three months—it may take some time to reach the optimum improvement—have maintained it as long as they have been observed. On the other hand, there have been patients who after an initially excellent response are not as well as they were.

Of 7 patients with an A type response to adrenalectomy who lived more than one year, four were in Smithwick group 4. One of them died at 15 months, and three are still living, indicating that even in the most severe hypertensives one can get this good result. Two patients were in Smithwick group 5, one in Smithwick group 2.

Figure 22 shows the course of S. C., the first patient in our series to have adrenalectomy for hypertension. This shows the response in a patient with a class A reaction. The high systolic and diastolic pressures in a control period and the normal blood urea nitrogen and the high normal urinary corticoids in this patient are recorded. After subtotal adrenalectomy, the systolic pressure fell fairly rapidly. It took two months for the diastolic pressure to come down from 130 to 70 or 80. The time required for this response deserves attention. The patient had a low corticoid excretion at the 6 and 12 month periods shown in the top part of the chart, and then there was a high corticoid excretion at 24 months. This was due to the fact that she became pregnant and was

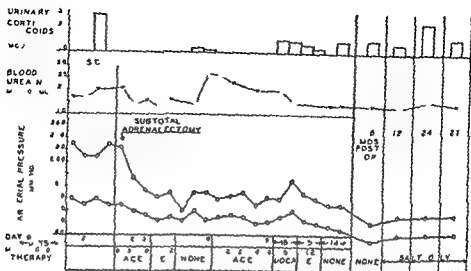


FIGURE 22 Patient S C Hypertension known for 13 months sympathectomy performed 9 months before subtotal adrenalectomy ACE = adrenal cortical extract (Upjohn) E = cortisone DCA = desoxycorticosterone acetate A portion of this Figure appeared in and is reproduced by permission from Lukens F D W and Wolferth C C Observations on subtotal adrenalectomy in hypertension *Tr Am Clin & Climatol* 4 62 229 (1950)

capable of having the increased corticoid excretion of pregnancy. It is interesting that this could occur in a woman who had a striking change in blood pressure. She went through a normal pregnancy without any adrenal substitution therapy and without any change in her blood pressure or any other unusual event. In not needing replacement therapy she is an exception to the general rule. At 27 months, she reverted to her usual corticoid excretion. Her blood pressure continues normal and she is fine.

Asst Wood: Was there a sympathectomy performed, too?

Lukens: Yes, she was one of the patients who had an extensive Smithwick's sympathectomy nine months prior to her subtotal adrenalectomy and the sympathectomy had failed to benefit her blood pressure as shown by the high initial readings (Figure 22). She is one of the patients who point up the fact that there is an adrenal cortical component in certain cases of hypertension just as Smithwick and others have demonstrated a sympathetic or neurogenic component of hypertension. It is difficult at the present time to appraise the relative importance of these factors in the patient.

Rall: How long had this patient's hypertension been present prior to the Smithwick operation?

Lukens Severe hypertension had been discovered four months before the sympathectomy and a year before adrenalectomy

Ralli Did her symptoms involve severe headache?

Lukens She had some headache. Patients can tolerate so many types of hypertension that it is a difficult problem to decide on surgical measures. There were no Smithwick group I patients in our operated series because those people have a long good prognosis. Our effort is to select the very severely hypertensive patient before the stage of renal insufficiency.

Long You just ventured the opinion that there was probably an adrenal cortical component here but during pregnancy when presumably the amount of let us say E and F present in the blood was greatly increased there seemed to be no effect upon the blood pressure.

Lukens Yes.

Long Is there any known adrenal cortical steroid which if given to this patient would bring the blood pressure back up? That it seems to me might be one of the best indications that one is dealing with an adrenal cortical component.

Lukens That is answered in a rather inadequate way in Figure 22. Fairly soon after operation this patient had 5 mg of DCA daily for a period of 18 days. That is a fairly large dose for an Addisonian although not massive. It can be seen that there was a tendency for the blood pressure to rise. We did not carry the experiment beyond that because we felt far more hesitant about doing things with these people then than we do now. We have not investigated this to any extent for we have been trying to reduce substitution therapy to get the blood pressure down in the patients who did not respond rather than trying to bring it up in those who had a good response. But we should do more such studies.

Ralli Would increasing the salt intake in these individuals affect their hypertension?

Lukens We haven't forced salt. We have given supplementary salt up to five grams a day for adrenal insufficiency to the patients in whom it doesn't raise blood pressure most of the time. I think we would probably agree with Perera (6) that salt alone does not have a hypertensive effect when the originally elevated blood pressure has been reduced by adrenal insufficiency.

Ingle Dr Lukens I recall seeing that patient within a few weeks after operation. At that time she was still very weak. Was she able to resume her usual household activities?

Lukens Yes she has resumed her full household activities including this pregnancy.

Thorn To comment on Dr Ralli's question I believe that what salt

does in an adrenalectomized patient in relationship to hypertension may vary with the renal status. If a patient because of renal disease is unable to retain salt within his body this may explain the apparent ineffectiveness of sodium chloride as a contributing factor to hypertension. However, it is not reasonable to exclude salt as a factor in the maintenance of hypertension in patients who retain appreciable quantities of salt. Certainly from clinical experience one would deduce that the level of hypertension may be increased in many individuals in conjunction with increased salt retention.

Lukens In that connection the effects of adrenalectomy and a regimen which might permit salt equilibrium at a different level should be related to the study reported by Dole (7) in which five patients had a very marked fall in blood pressure on a prolonged low salt intake. We are studying that now, hoping that some such test may differentiate the patients who will have the best type of response. We have nothing to report at present.

Dr. Selye referred to Green's paper (8) on post DCA hypertension. In that study Green tried adrenalectomy in hypertensive rats and it did not benefit the hypertension in these animals. But in the same journal Fitts (9) reported that adrenalectomy abolished the hypertension of animals made so by a cellophane capsule about the kidney. It is interesting to see in one copy of the journal this different response to adrenalectomy in two different types of experimental hypertension and to recall the fact that the patient likewise may or may not respond to adrenalectomy.

Rall Does the ketosteroid excretion in patients with severe liver disease depend on the compound that is given?

Thorn No, it is decreased with almost any compound we have tested. Would you support that, Dr. Conn?

Conn Yes.

Rall I mention this because the question arises whether some adrenal cortical factor is responsible for or contributes to hypertension or whether salt retention does so. Patients with cirrhosis of the liver retain salt and are unable to excrete it normally. Yet one practically never sees a patient with severe cirrhosis with an elevated blood pressure. As a rule the blood pressure is low. We have seen patients who were previously hypertensive and who following the development of cirrhosis of the liver have become hypotensive, yet at the same time these individuals retain salt. Apparently the retention of salt per se is not the cause of the hypertension or at least does not cause it when the secretions of the adrenal hormones are not increased.

Lukens We certainly don't understand the detailed relation of salt or salt retention to this process.

These experiences also raise the question of permissive action of the hormones because the best deduction I can make is that patients can maintain their blood pressure not only on a maintenance dose which could be permissive but on the total withdrawal of hormone up to the point of adrenal insufficiency. The blood pressure may even be elevated to the point of actual circulatory collapse in some patients.

Selye If I might add here also referring to your remark concerning Dr. Green's paper I think one has to differentiate very clearly the metabolic type of post DCA hypertension and that which may be caused by the continued action of mineralo-corticoids. We ran across this problem first in our work on the endocrine kidney. There adrenalectomy tended to abolish the renal hypertension but even maintenance doses of adrenocortical extract sufficed to re-establish it. Hence we concluded that in this case the hypertension was actually due to some renal pressor substance and that the corticoids merely exerted a conditioning effect in that they maintained the animals in a sufficiently good condition to allow the hypertension to manifest itself.

In animals which developed high blood pressure as a result of short DCA treatment interruption of that therapy reduced the blood pressure to normal and they did not enter into a state of corticoid insufficiency. Here the mineralo corticoid was apparently the primary cause for the maintenance of the high blood pressure level.

In the metacorticoid hypertension which is what Green was working on the rise in blood pressure persisted long after prolonged DCA administration had been stopped. In that instance the pressor effect apparently originated outside the adrenal (perhaps in the kidney) and the adrenal was not necessary for its maintenance as long as severe hypoadrenism did not cause too much systemic damage.

Lukens That raises a very important point as to the time in the course of the disease when adrenalectomy is worth while. Our experience is that the duration of the disease makes no difference. We have had good responses in people who have had the disease 10 or 12 years. This should be post DCA if that is a factor.

Selye It would be very difficult to be certain when the metacorticoid type of hypertension begins to develop and the adrenals are no longer of primary importance. This is probably a matter of dosage conditioning factors etc. What I meant to point out particularly was that although both types of experimental hypertension were primarily caused by an excess of DCA their mechanism and continued dependence upon corticoids is entirely different.

Lukens Quite different. We don't know what hypertension is in man.

Pincus Didn't Dr. Green report that hypophysectomy was effective in these animals?

Lukens Yes

Pincus You have not done a hypophysectomy?

Lukens We have discussed it but haven't done it. I had heard that Dr Olivecrona of Sweden had done hypophysectomy in hypertensive patients and lost them all.

Ralli Our present resident in neurology at Bellevue spent some time with Dr Olivecrona in Sweden and assisted him in some of the hypophysectomies in human subjects. He reported to me that their cases did very well. The only cases they lost were the malignancies that would have died anyway.

Selye I had an opportunity to look into the records of some of these cases operated by Olivecrona with my friend Dr Rolf Luft this summer in Stockholm. As Dr Ralli says at least those operated for carcinoma or diabetes were doing very well.

Thorn I have had correspondence concerning a patient in Buffalo who was hypophysectomized in an attempt to correct malignant hypertension. The patient died during the last postoperative period. Real difficulties may be encountered in the surgical removal of the pituitary in the absence of pituitary enlargement. It is not easy to carry out a complete hypophysectomy. It is a question whether or not patients who are so seriously ill as to justify hypophysectomy can in most instances survive one.

Ralli Apparently the Swedish neurosurgeon Dr Olivecrona is very skilled with this procedure and does it with considerable success.

Selye And may I say that as far as I could make out during my brief stay in Stockholm the operation is even less dangerous than that of bilateral adrenalectomy if it is performed under technically perfect conditions.

Thorn So much of the postoperative convalescence would depend upon the ability of the neurosurgeon and the clinical status of the patient upon whom the operation was performed.

Ingle Dr Lukens, do those patients who fail to have lower blood pressure following adrenalectomy have advanced arteriosclerosis?

Lukens One cannot point to that as the distinguishing factor because many of the patients who responded have equally advanced arteriosclerosis. We can't explain it at all.

Pincus Haven't you been conducting urinary steroid excretion studies with these patients?

Lukens Yes. A number of studies of urinary steroid excretion have shown that the hypertensive patient does not have any gross abnormality in steroid excretion and pending the development of new methods we are now using the few patients with total adrenalectomy to investigate the metabolism of steroids. We fail to find any abnormality with present methods.

Conn While we are discussing the question of permissive action of corticoids on hypertension and the possible relationship of this phenomenon to the sodium ion we might consider the hypertensive who develops Addison's disease. Such patients then become hypotensive with their Addison's disease. Administration of desoxycorticosterone restores the hypertension. We have had the interesting experience of having such a patient who without any desoxycorticosterone but with cortisone alone, in amounts far too small to produce significant sodium retention, developed severe hypertension. That hypertension was produced by 20 mg of cortisone a day and that patient's blood pressure can be regulated at will by reducing the dose of cortisone to 15 to 10 or getting up to 25 mg a day orally. Thus there are other problems involved besides sodium alone or the hypertensive effect of desoxycorticosterone alone.

Lukens There is no doubt about that. We have been aware of the fact that cortisone may have a hypertensive influence. We haven't studied it as accurately as you.

Pincus Did you use corticosterone in that patient?

Conn No.

Lukens We have seen a number of patients who were given 25 mg of cortisone a day and maintained a normal blood pressure and a number who needed 25 mg of cortisone a day but maintained a hypertension. Thus there is still a problem of differentiation.

Astwood Since in some patients one can give complete replacement therapy with cortisone and have a good response as Dr Lukens points out the permissive action in Ingle's sense is not sufficient to explain the response. Another possibility is that the adrenal may be making some other steroid which contributes to the hypertension. Could that not be tested by giving cortisone to cause adrenocortical atrophy as Wilkins and others have done in cases of the adrenogenital syndrome before taking out the adrenals to see whether there is a response?

Lukens We have watched the work of Dr Wilkins (10) with great interest because as you know he included the suppression of hypertension in a patient with virilism by giving cortisone. But with the few hypertensives that we have tested, a half dozen or so, we have never been able to lower blood pressure by giving cortisone. We had hoped that that would be a diagnostic procedure. Perhaps we should try it more extensively.

Long How much cortisone have you given?

Lukens Well in one case we gave 200 milligrams. That may have been too much.

Thorn Yes.

Lukens In some we have given smaller doses. But we have never

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It has been interesting to observe that before the renal impairment becomes too great a significant improvement in the clinical condition can be brought about by bilateral adrenalectomy. These patients rapidly lose their edema and are able to enjoy an unrestricted diet. In one patient in this category who required sodium restriction before operation and who following operation was able to eat an unrestricted diet we tested the effect of desoxycorticosterone and cortisone. On 37.5 mg of cortisone this patient could not be kept in mineral balance without supplementary salt intake. On the other hand desoxycorticosterone re-produced her cardiac enlargement and hypertension as did corticosterone. Desoxycorticosterone was effective in this regard in less than 1 mg per day and corticosterone in a dose of 50 to 100 mg daily.

Lukens This was a nephrotic?

Thorn This patient had had a long standing nephrotic syndrome but had within the past year developed severe hypertension with headaches.

There is another point regarding the susceptibility of the hypertensive patient to hormone withdrawal. These patients have never previously experienced a complete withdrawal of adrenal cortical hormone from their bodies and in contrast to the patients in whom Addison's disease has developed slowly are extremely sensitive to the complete removal of cortisone. In such patients a crisis can be precipitated in forty eight hours if all hormone therapy is suddenly discontinued. In regard to the patient I have been speaking of we observed that on a maintenance dose of 37.5 mg of cortisone per day 1 mg of desoxycorticosterone every other day produced cardiac enlargement pulmonary congestion and the hypertension which was present prior to operation. By discontinuing the 1 mg of desoxycorticosterone and adding 50 mg or 100 mg of corticosterone to the basic maintenance dose of 37.5 mg of cortisone we were able to reproduce the same changes as those with desoxycorticosterone. I have no doubt but that Dr. Conn is right and that perhaps in rare instances even a dose of cortisone as small as 25 mg may be sufficient to tip off this mechanism in some patients. In the majority of patients however the Addisonian maintenance dose of cortisone 25 mg per day does not appear to be effective in restoring the changes that were modified by adrenalectomy. On the other hand if one attempts to suppress endogenous adrenal hormone secretion in these individuals with 100 to 200 mg of cortisone per day the inhibition of secretion of desoxycorticosterone like factors might be more than offset by the hypertensive effect of the larger doses of cortisone.

Bush The dosage of 37.5 mg of cortisone and either 6 grams of salt or 1 mg of DCA didn't restore hypertension?

Thorn No. That is 1 mg of desoxycorticosterone acetate every other day in addition to the cortisone but without supplementary salt the

seen a patient with essential hypertension respond the way Wilkins patient with virilism did

Thorn Dr Perera reported on his observations some years ago. He used, I believe, 100 mg of cortisone per day. I should think that 50 mg per day particularly by injection would be more nearly the correct dose. In commenting on Dr Lukens' results it would appear to me that his group does much better therapeutically with the combined operations than when bilateral adrenalectomy alone is carried out. In part this may be due to the more pronounced changes in blood pressure particularly in the upright position. These changes occur of course with sympathectomy but are not often seen in our patients following bilateral adrenalectomy.

Lukens Our clinical impression agrees with that.

Thorn There is a very good possibility of that being an important element in your results. The second point which I should like to discuss is that in some of the patients we have studied it is our impression that the important modifying influence of the adrenal in hypertensive vascular disease is almost completely lost when advanced renal complications are involved. As renal disease progresses the modifying influence of the adrenal hormone appears to be less and less.

A third point is that in hypertension and particularly in cases of chronic pyelonephritis when total daily sodium chloride intake is limited to five or six grams and the patient becomes depleted of body sodium and chloride reserves we have taken this as a contraindication for adrenalectomy because we feel that one of the specific benefits which patients have derived from bilateral complete adrenalectomy is that they can eat an unrestricted salt diet. Thus if the hypertensive vascular disease is a type which does not require salt restriction we are less enthusiastic about undertaking adrenalectomy. Ideal cases for complete adrenalectomy consist of those associated with marked hypertension and congestive failure or excessive salt retention where there is reasonable renal function. Instead of constituting a contraindication I feel that cardiac failure in the presence of reasonable renal function is an indication for bilateral adrenalectomy. Our trouble is much the same as that outlined by Dr Lukens in that radical procedures at this point at least must be reserved for patients with advanced disease. On the other hand when the disease has advanced too far none of these procedures is particularly helpful.

Another group in whom we have become interested is characterized by the middle aged patient with the nephrotic syndrome who having gone along for years with edema, hypoproteinemia and normotension insidiously begins to demonstrate hypertension. With the onset of hypertension in our experience these people run a rapid downhill course.

and immediately following operation and that part of their immediate rehabilitation is obviously a cortisone nonspecific effect

Whereas the dose of cortisone used in most of these patients is higher than that used for Addison's disease maintenance therapy it has been the consensus of opinion that desoxycorticosterone is not necessary for many of these patients. Thus it would appear that when because of cancer adrenalectomy is carried out a better balance is established by giving relatively larger doses of cortisone and smaller doses of desoxy corticosterone than in the case with patients with spontaneous adrenal insufficiency

Lukens Congestive failure may well be an additional indication for operation because the improvement in patients with congestive failure due to hypertensive disease has been remarkable. Men who have had two or three bouts of congestive failure have been able to resume active work after adrenalectomy something that could not have been accomplished by digitalis in the majority of instances

In addition to the striking benefit in congestive failure there has been a significant improvement in hypertensive retinopathy. This improvement comes late after a year at the earliest at eighteen months more commonly

We have not observed any significant changes in kidney function. The few studies that have been done by Drs. Clark, Crosley, and Barker (11) show that kidney function was about the same that renal plasma flow was about the same. As this occurred at a lower level of blood pressure it meant that renal resistance had changed. Similarly it has been determined that blood flow is the same in the brain at the lower pressure (12). Supplementary studies of this kind have been and are being made as we go along. The matter of judging how much adrenal remnant there is and what response will occur is now actively in progress. Dr. Hills is using the various eosinophil response tests that you have outlined. Dr. Thorn

Long If subtotal adrenalectomies are done in the rat or the cat it is not very long before regeneration takes place and the same amount of cortical tissue as was present pre-operatively is found. In cases of subtotal adrenalectomy in man are we to suppose that the rate of regeneration in the human adrenal is markedly different?

Lukens I suspect that the regeneration differs in different patients. Two or three of our patients were re-operated to have subsequent sympathectomy because their blood pressures did not fall. At that time an adrenal remnant was removed and in all instances where that last remnant was removed one could see the difference in their need for therapy whether or not their blood pressure responded

Sayers How large was the remnant?

equivalent of 0.3 mg of desoxycorticosterone per day. A dose of 0.3 mg of desoxycorticosterone every second or third day was as much of this hormone as the patient could tolerate without developing undue signs of salt and water retention.

Conn: Did you attempt to find a ceiling for cortisone alone?

Thorn: No, we haven't done that. I would expect that a ceiling for cortisone would be soon reached if the dose went much beyond 50 to 100 mg daily.

It might perhaps be asked how we prove complete adrenalectomy. Since most of these patients are on cortisone replacement therapy for long periods of time, intensive ACTH stimulation must be given before one can assume that there is no residual adrenal cortical tissue. In general, we have relied on 3 successive 8 hour intravenous infusions of 25 units of ACTH each, given on successive days. We study the response not only of the eosinophils but of the 17 ketosteroids and 17 hydroxy steroids in the urine following this intensive period of ACTH stimulation. We are not satisfied with the interpretation of complete adrenal ectomy unless our values show no adrenal activation under these circumstances.

There is one final point I should like to mention in regard to the discussion of bilateral adrenalectomy in the case of patients with prostatic cancer and metastases. For some of the group who are not familiar with the clinical aspects of this procedure, the theory behind adrenal ectomy after castration was that there might be androgens secreted by the adrenals which would be favorable for the prostatic growth. It was felt that by removing the adrenals completely it would be possible to eliminate this source of adrenal androgen. In general, I think the results have tended to confirm this in a high proportion of patients who thus far have been subjected to bilateral adrenalectomy. There is the point, however, that cortisone replacement therapy does provide at least some biologically active androgen in the urine, although the quantity involved may be smaller than that initially present prior to adrenalectomy. For a matter of record, I should like to state that very few biological androgen studies have been made on the urinary excretion of patients with prostatic cancer before bilateral adrenalectomy and following bilateral adrenalectomy on cortisone therapy. However, there appears to be no doubt but that the maintenance dose of cortisone used by most observers, 50 mg per day following bilateral adrenalectomy, has an over all beneficial effect on the patient.

The second point to be considered of course is that although the interpretation of improvement might be on the basis of reduced circulating androgen in these patients following adrenalectomy, it must also be realized that these patients received large doses of cortisone during

arterial disease? In diabetics the degenerative lesion is one of the most depressing aspects of the disease. One almost feels as if the carbohydrate disturbance is in the nature of a red herring drawing attention away from the fact that in essence the disease involves severe degenerative lesions of the small arteries. Patients with diabetes would seem to me to constitute an ideal group in whom to do complete adrenal ectomy. There is also the question of whether the retinopathy and arterial lesion in the diabetic is associated with an overproduction of adrenal cortical hormones or a sensitivity of the tissues to these hormones. At times one feels that diabetes is perhaps primarily a disease of the adrenal cortex and that the pancreas is involved only secondarily.¹

Lukens It is commonly accepted now that diabetes has a metabolic component and some other component which we vaguely name degenerative. As for doing adrenalectomy in such patients I have been wishing we could. We have not encountered the appropriate patient yet.

Long They should not be hard to find.

Ingle Dr Green's patient who was adrenalectomized had severe diabetes and hypertension. Vision was seriously impaired. The eye grounds improved and the diabetes and hypertension were ameliorated following removal of the second adrenal.

Lukens Yes that one improved and I can cite another. Last summer Dr Paulsen of Denmark reported a patient with diabetes and advanced retinopathy who became pregnant and developed Simmonds disease and the diabetic retinopathy completely disappeared.

Long Along with the diabetes?

Lukens The diabetes was modified but it did not completely disappear.

Thorn While on paper this would appear to be a desirable group to work with we have all had some experience in the management of severe diabetes in an adrenalectomized patient and when complicated by hypertension this is not so simple.

Lukens No it is exceedingly difficult and the only reassuring thing there is that most of the middle aged diabetics with hypertension probably do not have severe diabetes. Less would be learned about diabetes but a more successful result should ensue than if the diabetes were severe.

Ralli Am I to infer that these patients are very sensitive to insulin?

Lukens We have not tested their response to insulin. If they have adrenal insufficiency I presume they are sensitive to insulin.

Thorn We have had several patients with Addison's disease and diabetes and the manipulation of cortisone and insulin is very satisfactory in these patients most of the time. However there has not been the added factor of hypertensive vascular disease which adds a worri

Lukens That varied in different instances. The last one removed was an ample adrenal remnant that obviously could support life.

Sayers This remnant had no medullary tissue?

Lukens No.

Sayers It was entirely cortical tissue?

Lukens Yes. The small amounts of adrenal cortex left would be far too little to contain any medulla.

Long Had there been appreciable growth between the two operations?

Lukens That could not be determined because of scar tissue. However, in one instance it certainly was larger. I think the real answer to your question is that many patients with subtotal adrenalectomy who require substitution therapy have required it for as long as they have been followed. There is no regeneration in the sense that they escape the necessity for replacement therapy.

Ingle In the rat, extensive partial adrenalectomy can prevent the remnant from regenerating to normal size although it does undergo considerable hyperplasia.

Lukens Many people who have adrenal insufficiency after subtotal adrenalectomy have a margin of reserve. If they stop taking replacement therapy instead of going into crisis in one week, it may take three or four weeks. There is a small factor of safety which is not to be disregarded.

Long The next question is whether this apparent slowness of regeneration of the remnant is related to the fact that in almost all instances substitution therapy has been given.

Lukens No. I don't believe that is the explanation. Dr. Long, because we have always tried to give minimal supportive therapy in an effort to keep the blood pressure down. The only exception to that is the fact that in the immediate postoperative period we have had to give ample therapy to get the patients well and out of the hospital. If that stops regeneration, then we have done it at that time but not later.

Conn One might have a partial answer to your question, Dr. Long, in those patients who have had subtotals for Cushing's syndrome where substitution therapy has not been added and where they have been clinically cured.

Lakers That is true, Dr. Conn. There are two types of patients: the Cushing syndrome that has been totally cured and those that have recurred as a result of regeneration. One cannot say in a single patient how much the adrenal remnant will regenerate.

Rall Have you had the possibility of doing this operation in patients with diabetes mellitus, particularly those with diabetic retinopathy and

adrenalectomy? Did the cause of their hypertension lie in the adrenal glands or was it extra adrenal? It seems possible that in these patients the cortical hormones played only a permissive role in supporting the hypertension

There is another point which comes to mind a remark which I heard Dr Corcoran make a few years ago He said in effect There is something about being debilitated from any cause which is incompatible with the manifestation of hypertension not an exact quotation but the gist of it I wonder whether the relationship of the adrenal cortex to hypertension is more specific than this?

Long Dr Ingle I have often thought of this question in relation to your work on skeletal muscle where the asthenia as a result of the deprivation of cortical hormone is so very marked Might it not be that in adrenal insufficiency there is a situation in the smooth muscle of the blood vessels that is comparable to the asthenia that you so readily observed in the skeletal muscles? If this is so there might be relaxation of all the smooth muscles that go to make up the arterial tree

Thorn Except that patients with Addison's disease are known to be quite sensitive to pressor substances

Ingle Recent studies by Levine and his associates at Michael Reese Hospital may have a relation to that It was found that when mesenteric vessels of the adrenalectomized rat become unresponsive to nor epinephrine the responsiveness can be restored by the topical application of cortisone This group feels that the muscular asthenia of the adrenally insufficient animal is based upon circulatory failure We had made this suggestion years ago without direct evidence for it

Dr Lukens can you make a discrimination between the effects of adrenalectomy in hypertensive patients and the effects of injecting pyrogen?

Lukens First of all the injection of pyrogen would have short term effects These patients live in a steady state for months Another distinction is that the level of daily life and social usefulness of most of these people could not be supported if they were in a debilitated condition So that while a general debilitated condition must be considered in one's thinking the level of that debility is rather mild

Sayers Dr Lukens you said something about the ability of these bilaterally adrenalectomized patients to maintain their blood pressure after withdrawal of replacement therapy Would you go into a little more detail on that? Am I correct in saying that when replacement therapy is withdrawn the hypertensive bilaterally adrenalectomized patient will maintain a higher blood pressure than a patient with Addison's disease whose replacement therapy has been withdrawn?

Lukens Yes If a patient with a very high blood pressure has had the

some factor regarding hypoglycemic reactions. It would appear in the group of patients we have studied thus far that adrenalectomy cannot be claimed to cause regression in the vascular changes, with the exception of changes in the fundi which appear in many instances to be ameliorated if there is reduction in the blood pressure by any means. One of our best results was obtained in a young man who had been disabled for several years because of his hypertension and cardiac changes. He returned to full activity for over a year but died suddenly, approximately 15 months after total adrenalectomy. Autopsy showed widespread coronary artery disease. There seemed to be no evidence in this individual of any regression of his vascular changes.

Lukens Most of our patients who have had angina pectoris are surprisingly little relieved. Those who have had congestive failure are much relieved. And that distinction may be of some importance to our thinking.

Selye I should like Dr. Ingle to comment on the role of the adrenal in this kind of hypertension if he will. In particular for instance if both adrenals are removed and the patient maintained in good condition on the necessary corticoid therapy and it is seen that, despite the adequate corticoid treatment the hypertension does not return, how do you interpret this? Would you feel that fits in with your concept of the purely permissive action of corticoids in this disease? Furthermore when a subtotal adrenalectomy leaves only a small remnant just sufficient to maintain the patient in apparently good health but insufficient to maintain a pathologically high blood pressure level, how does that fit in with your concept of a merely permissive action? Or wouldn't you apply your theory to this kind of case?

Ingle I was more willing to apply it before this Conference began than I am at this point.

Likens I think in some instances there is no permissive action and in others there might be permissive action at very different levels in different patients which raises a special question for the investigator.

Ingle I have thought that the cortical hormones play a permissive role in the maintenance of experimental renal hypertension. Renal hypertension is ameliorated by adrenalectomy and yet the exciting cause of the hypertension is extra adrenal. The fact that it is not fully manifest in the adrenally insufficient animal seems to me to be an example of permissive action.

Drs. Lukens and Thorn have found that in some patients the production of adrenal cortical insufficiency fails to lower blood pressure. In such cases it would not seem that the adrenal cortices have played either an etiologic or a permissive role in the hypertensive state. But what should we say of those patients whose hypertension is ameliorated by

adrenalectomy? Did the cause of their hypertension lie in the adrenal glands or was it extra adrenal? It seems possible that in these patients the cortical hormones played only a permissive role in supporting the hypertension

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Lukens Yes. If a patient with a very high blood pressure has had the

adrenals removed and if that patient does not respond and the hypertension continues then even on withdrawal of all replacement therapy the hypertension continues up to the point of circulatory collapse & then obviously it goes down

Conn But that is the time when adrenal steroidal activity ceases isn't it?

Lukens One can very well argue that point. It is difficult to determine at what point all the cortical hormone disappears.

Conn But when the patient has that vascular collapse and is given some cortical hormone the blood pressure comes up again.

Lukens That is right.

Conn So that as far as permissive action is concerned I think no conclusive evidence has been presented today against it.

Lukens Not if you recognize the fact that that permissive action is taking place at different levels of adrenal hormone. In other words of an imaginary 10 patients on the same maintenance therapy 5 have a normal blood pressure and 5 have hypertension so that the permissive effect of that particular dose is not uniform.

Selye Would Dr. Ingle agree to using his term in the sense of envisaging permissive actions occurring at various dose levels? If we are going to say that a certain action is permissive and yet can occur at various dose levels then all physiologic phenomena can be visualized as permissive. I should have thought that the term could only be used for yes or no responses as originally suggested by Dr. Ingle i.e. a factor either does or does not permit an effect to occur but never alters the degree of that effect. In a dose response relationship then I think the term permissive in its original formulation can no longer be applied.

Lukens Well not as rigidly as one does in the laboratory.

Loewi Speaking only of the physiologic actions of adrenocortical hormones not of their pharmacologic actions what is meant by permissive action? In 1933 a very important paper was published from Dale's laboratory (13). The authors observed that starving rabbits died from hypoglycemia one or two days after hypophysectomy although the liver and muscles contained a lot of glycogen. The authors concluded that the animals apparently could not mobilize glycogen. To prove the correctness of this view they injected such animals with epinephrine; it failed indeed to mobilize glycogen. Later they found and this was confirmed by others that after hypophysectomy mobilization from the stores of fat is inhibited too. This inhibition occurs because in hypophysectomized animals cortical hormones are no longer secreted. Thus the mobilization of fat is inhibited also in adrenalectomized animals. Finally it was shown that mobilization of protein is also inhibited under the same conditions. If the mobilization of protein was not inhibited

in starving hypophysectomized or adrenalectomized animals the blood sugar would not drop. It does not drop in starving normal animals as they are capable of mobilizing protein for the formation of glucose. From all these results it became obvious that the adrenal cortex is needed for the mobilization of foodstuffs.

Other physiologic actions of the cortex were demonstrated by Ingle. He showed that after adrenalectomy stimuli which in normal animals produced special effects were no longer effective. Among such stimuli were fracture of bones, asphyxia and cold. However these stimuli were effective in adrenalectomized animals if they had received physiologic doses of cortical hormones.

I came to the conclusion that the physiologic action of cortical hormones consists of maintaining the normal responsiveness of organs to metabolic stimuli. Why is this called a permissive action? The ability of insulin to maintain the normal blood sugar level is not called a permissive action. It is a physiologic action. I don't see any reason why one should call the action of the cortical hormones a permissive rather than a physiologic action.

Long I raised this same question earlier because I have had certain doubts on the semantics myself. What is the difference if there is a difference between a permissive and a physiologic effect? I can recognize the effect of no hormone at all and I can recognize the effects of an excess. But under physiologic conditions presumably the amount of cortical hormone is placed somewhere between those two extremes.

Thorn I too have some questions. I believe that most of us are using 25 mg. of cortisone per day in the hypertensive patients following complete adrenalectomy.

Lukens That is a common dosage.

Thorn Does 25 mg. of cortisone in hypertensive patients represent more or less than these patients secreted prior to their adrenalectomy? This may be important because in grading how a patient feels we know that if the cortisone dosage is greater than his own adrenal was customarily putting out prior to operation part of his improved sense of well being may be the so called nonspecific effect of cortisone and might he not feel better on 25 mg. of cortisone with his adrenals intact. This is something we must think about and may need to evaluate subsequently.

A second problem relates to studying these patients with smaller doses of hormone or with no hormone whatsoever. We know that the sudden withdrawal of hormone can precipitate a crisis. On the other hand we might reproduce the spontaneous development of Addison's disease by very slowly reducing the hormone over a prolonged period of time and in this way see if the individual's system would adjust to

small quantities of adrenal steroids. If sufficient sodium chloride is provided I am certain it would be possible for these patients to get along on very low levels of hormone. I doubt whether the patient would feel as well as he does under cortisone therapy, but it would provide an opportunity to explore Dr Ingle's concept of the permissive aspect of adrenal cortical hormone therapy.

Ingle Dr Thorn, are there any adrenalectomized, hypertensive patients whose blood pressure cannot be reestablished at the preadrenalectomy level by the administration of cortisone alone?

Thorn I can't say because we haven't given them unlimited quantities of hormone. Certainly 25 to 50 mg per day does not restore the preoperative signs and symptoms of many of our patients.

Ingle Here is a very basic question. Does adrenalectomy remove an agent which has caused the development and maintenance of hypertension?

Lukens I don't know anything about the cause of hypertension.

Ingle Would you venture a guess as to what the role of the adrenal cortex may be in the etiology of hypertension?

Lukens It is more likely to be supportive than causative.

Long Yet a fair number of individuals with Cushing's syndrome have hypertension; do they not?

Lukens The majority of them.

Long So that an excess of something from a human adrenal will cause hypertension and removal of the tumors or the gland or perhaps the pituitary itself will relieve it.

Lukens I think both Cushing's syndrome with its hypertension and the response of some patients with hypertension to adrenalectomy suggest that the adrenals may be an important factor in either the cause or the maintenance of the hypertension in a limited number of instances. But limited number of instances must be emphasized because certainly there are many forms of human hypertension that seem to be independent of that mechanism or relatively independent.

Ingle One might also say that the same is true of the relationship of the adrenal cortex to diabetes. There are a few in which it is clearly a factor.

Have you formed any opinion which you would care to express, Dr Thorn, as to the relationship of the adrenal cortex to the etiology of hypertension?

Thorn I would be willing to say this: in the majority of people whom we see with advanced hypertensive vascular disease, the most important single factor is probably not the adrenal cortical hormones; second, the adrenal cortical hormone is certainly a factor in the maintenance of hypertension and particularly is it important before the onset

of high grade renal insufficiency. However, once renal insufficiency is well established, there would appear to be less relationship between the absence of adrenal cortical hormones and the maintenance of hypertension.

Sayers In Cushing's syndrome, isn't it true that in a large percentage of these patients there is some renal damage? What relationship does that renal damage have to the hypertension that develops? I read a review by Eisenhardt and Thompson (14) in which a large series of these patients came to autopsy and if I remember correctly, about 50 to 70 per cent had a fair degree of renal damage. I am interested in what comes first, the renal damage or the hypertension.

Thorn Throughout the United States in the past year or two, there must have been a large number of patients in whom Cushing's disease was induced with excessive cortisone therapy. As far as I know, the incidence of hypertension is relatively small. It is my impression that the percentage of hypertension in patients with spontaneous Cushing's disease is much greater, although this would certainly need statistical analysis. Whether or not such statistics would be helpful in elucidating the point that the total adrenal secretions are more conducive to hypertension than cortisone alone remains to be seen.

Long Have you produced Cushing's disease in the strict sense of the term with cortisone? My impression from reading the literature is that in a large group of people treated with cortisone, some develop hypertension, some the moon face, and some hirsutism, but that nobody so far has gone to the extreme of continuing the cortisone once the first sign of excess dosage appeared, so it is quite different from the natural disease.

Thorn We have continued cortisone in the face of signs of excess dosage when very severe underlying disease has been present and there seemed to be no other means of helping the patient. These patients have then been maintained with obvious Cushing's syndrome for fairly prolonged periods of time.

Long That would be the group to compare with the natural disease.

Pincus I believe that Dr. Sprague, in his comparison of the effects of cortisone in Cushing's disease, found practically every symptom, with I think two minor exceptions.

Long But not all in the same individual.

Pincus That is true.

Thorn Not all the patients with Cushing's syndrome have all of the manifestations of the syndrome.

Conn No, that is right. About 30 per cent of them don't have diabetes.

Thorn And some patients do not have hypertension.

Lukens To return to your question Dr Sayers, there is no doubt that there is a renal component in the hypertension of Cushing's syndrome at least in its later stages but in citing autopsy statistics one presents the worst possible picture as far as the kidney goes. Many of these patients at an earlier stage had clinically normal renal function.

Ingle What is the incidence of hypertension and of diabetes among patients with gigantism and acromegaly? I am thinking of the clinical implications of Dr Seley's recent studies with growth hormone.

Ralli Cushing and Davidoff (15) reported an incidence of diabetes of about 75 per cent in patients with acromegaly. We have observed three patients with acromegaly all of whom had diabetes interestingly enough never complicated by ketosis. The patients did have elevated blood sugars and elevated blood cholesterol. The one that is still living a male does not have hypertension.

Lukens Figures in the literature range from 12 per cent to the high frequency you mentioned.

Thorn Do the stigmata of acromegaly persist after the active phase of acromegaly has disappeared?

Lukens That is true.

Thorn It is a little difficult to evaluate the absence of diabetes under these circumstances.

Lukens I think it is generally agreed that acromegaly does not produce an increased incidence of hypertension.

Long So far as diabetes in acromegaly is concerned the statement has been made—it may have been made by Dr Cushing—that since the course of the disease is long, the patient is frequently seen in the so called burned out stage. Probably all acromegalics have glycosuria at some time during the disease.

Lukens That could well be.

Ralli We had one patient who had acromegaly and did develop hypertensive retinopathy. This patient died of a cerebral hemorrhage and at post mortem a pituitary tumor was found which showed both eosinophilic and basophilic cells. There has been a good deal of discussion about the amount of hormone that is needed by adrenalectomized patients but I have not heard whether when these people return to ordinary everyday life the need for hormone goes up. Suppose the man goes back to his work of brick-laying or whatever it is and he has been stabilized on 25 mg. of cortisone a day is there any indication that he must be given more cortisone?

Lukens It is difficult to answer that because we are constantly adjusting our treatment to these patients' needs. I might mention that the first hot summer our patients went through we were watching them all the time and we had one or two minor accidents but the last

two summers we had no extra visits to the clinic by any patient

Long And you have not had to raise the hormone?

Lukens We have not had to raise the hormone appreciably. That goes for intercurrent respiratory infections or their usual occupations. I am sure however that if we had the hormone especially adjusted at some very low level we might have to raise it. It may be that we are not measuring the thing accurately enough to bring out the answer to your question.

Long Dr Thorn raised this point a few moments ago when he was asking the question as to whether 25 mg was too much or too little. The experience seems to be that roughly 25 to 37.5 mg a day which were the figures you were quoting is ample in this series of patients although of course I have no information as to what their occupations were. But you say they go through intercurrent infections without the necessity of the hormone being increased?

Lukens Yes.

Long Certainly the Addisonian on 25 mg of cortisone would require more if he had an intercurrent infection.

Conn That is true in our experience. We have had very frequent experiences in which patients who had been maintained on 20 to 25 mg and 1 mg of DCA have had to be increased to 35 or 50 mg of cortisone usually not above 50 in the face of intercurrent infection.

Lukens We have had one or two crises precipitated by more severe intercurrent infections. It is hard to define intercurrent infection.

Thorn The problem partially revolves around the inability of the Addisonian patient or the adrenalectomized patient to compensate for physiologic or pathologic changes that are induced by a particular infection. For instance the presence of diarrheal disease results in the loss of a large amount of sodium chloride and water and under these circumstances it may be essential to give a very high level of adrenal substitution therapy whereas a patient could engage in heavy physiologic activity per day such as walking or chopping wood without perhaps the necessity for such a marked increase in his hormone requirement. It would appear that the patient with Addison's disease or an adrenalectomized patient can tolerate physiologic increases in work with only a small increment in hormone whereas pathologic changes such as dehydration may require much greater adjustment.

Long You say that a man balanced on 25 mg a day in the hospital can go out and chop wood. That means he can raise his basal oxygen consumption from 250 ml a minute up to say 3 liters a minute a very large increase in the metabolic rate. Nevertheless so far as you know there is no indication that there is anything like a proportional increase in the requirement of cortical hormone. The question of utilization has

come up here time and time again and it is important in understanding the action of the hormone. It seems to me that the adrenalectomized human affords us an excellent opportunity to find out exactly how much greater is the need for hormone within the physiologic range. Exercise may be one of the best ways to test it.

Selye Another aspect of the same problem is the role of the adrenal cortex in adaptation. Our first animal experiments which served as the basis of the adaptation syndrome concept relate to this point, one which is also important if we consider what Dr. Loewi said a few minutes ago about the role of the adrenals in maintaining a physiologic responsiveness of the tissues. In those experiments we showed that if a rat is previously adapted to perform violent muscular exercise to live in the cold or to be treated daily with a certain drug, it will retain much of its increased resistance even following subsequent adrenalectomy. Animals adrenalectomized prior to acquiring adaptation to such stressors have of course a very low resistance to them. It was somewhat unexpected that continued hyperfunction of the adrenal cortex was not necessary for instance to transform protein into sugar as a fuel for intense muscular work once adaptation had been acquired or to maintain the body temperature through metabolic processes despite exposure to cold. However that is what we found. Adrenalectomized animals even if maintained only on sodium chloride did preserve a great deal of their acquired resistance to all these stressors after adrenalectomy.

It is hardly possible to select any one biologic phenomenon as being the only one for which the corticoids are necessary. However it does seem to me that the above experiments show that the adrenal is not so much required for maintaining a responsiveness of the organs (after adaptation the organs appear to remain responsive to certain stimuli even in the absence of the adrenals) but for the acquisition of adaptation.

Long That brings us back to a point discussed before. What you say about the adrenal may be perfectly true but I think the phenomenon involves a great deal more than the adjustment to the adrenocortical secretion.

Selye Oh no doubt. What I meant to show by these experiments was that during the initial stage of the G.A.S. (the alarm reaction) when the organism first meets with circumstances to which it has not been adapted it needs a great excess of corticoids. Later during the stage of resistance it needs much less. I would think that this is so because the peripheral cells—in the case of adaptation to muscular exercise the muscle cell itself—can go on doing work quite efficiently with a minimum of corticoids if the organism had produced large enough

amounts during the initial stage while adaptation was being acquired

Long Couldn't you make out almost the same case for the thyroid hormone? That in adaptation to cold large amounts of thyroid hormone are required

Selye Has it ever been shown that an individual previously adapted to cold requires less thyroid hormone to resist cold subsequently and can do so even after thyroidectomy?

Long I don't know

Lukens In connection with adaptation these patients are hospitalized for a fairly prolonged period so that adaptation to life outside the hospital is slowly achieved. They are not subjected to a sudden test in that sense

Sayers Do you return these patients to an active life as soon as they get out of the hospital? For instance would a chap who has been active as a bricklayer go back to his occupation as soon as he is out of the hospital?

Lukens No and the chief reason is the orthostatic hypotension that most of them have. It takes two or three months for them to adapt to that

Thorn Our patients with complete bilateral adrenalectomy alone do not experience orthostatic hypotension

Sayers And you have seen a number of bilaterally adrenalectomized individuals do a considerable amount of muscular work without getting into difficulty?

Lukens Yes I would say so

Thorn We had one patient who had been disabled for over two years with hypertensive cardiovascular disease who within a month or two after his bilateral adrenalectomy was carrying on hard physical work

Selye Was this a person previously accustomed to muscular work of the same kind?

Thorn This patient was a well developed young man but he had been a complete invalid for two years. He had been very active earlier in his life

I would like to ask the group to interpret a phenomenon that we have observed of a marked fall in eosinophils following complete bilateral adrenalectomy in a patient who was given a constant large dose of adrenal hormone throughout and following the operation. With a constant intravenous infusion of compound F there was an eosinopenia followed by an eosinophilia. The interesting aspect of this experiment is that with continuing constant infusion of hormone in a patient without adrenals there was an eosinophilic rise to the pre-operative level

Long That is the question I raised previously if an adrenalectomized animal was given a constant amount of hormone and exposed to stress would the eosinophils fall?

Thorn The quantity of hormone given during the operation in this instance could explain the fall in eosinophils. However the magnitude of fall was similar to that seen spontaneously in patients with intact adrenals subjected to major surgery.

Long They follow the same curve on a constant amount of F?

Thorn After the first day of operation the patient was kept on a constant quantity of hormone. The eosinophil response seemed to break away from the constant infusion of hormone at a time when one would expect the hormone to be in excess of the patient's needs. We ourselves have been in error in interpreting this rebound in the patient subjected to surgery with intact adrenals as necessarily reflecting a state of adrenal inactivity or relative insufficiency of hormone. I would doubt that on the seventh day or eighth day with 100 mg. of F having been injected intravenously around the clock that one could assume adrenal cortical insufficiency was present at that time.

White This individual had what clinical state?

Thorn The patient I am reporting on was a hypertensive individual subjected to bilateral complete adrenalectomy.

White Is there evidence that on the seventh day the individual was still under what could be termed stress? Also why are we surprised by this rebound phenomenon?

Thorn We were surprised to see the rebound eosinophilia with the high level of hormone administered at this time.

Selye The statement has been made by several investigators that after adrenalectomy stress often tends to cause an eosinophilia. Preliminary experiments performed at our institute suggest that STH may counteract the eosinophilia normally produced by cortisone. One wonders whether endogenous secretion of STH may be involved in such responses. Has anybody here evidence on this type of action either in animals or in man? This would seem to be one possible compensatory mechanism designed to maintain a normal blood eosinophil level.

White The situation is one in which adrenal corticoid steroids are undoubtedly being used for purposes other than the production of an eosinopenia and the requirements of the tissues for adrenocortico steroids for these processes may influence how much of the adrenocorticosteroids are available for affecting eosinophils. That is why I asked whether the individual at the seventh day was still under a stress. If so his tissue utilization of hormones might be larger than supposed. The situation might be compared to the fact that in every circumstance in which a lymphocytopenia results there is set into motion various factors about which we know very little to restore the lymphocytes to normal. As a result of this production of new cells which are being made to replace cells that have disappeared there is invariably a re

bound phenomenon perhaps a reflection of a sudden rush of production

I think there are three factors first what the hormone does to the cells being counted which are disappearing under the hormonal influence second how much hormone there is for this specific function and third the rapidity with which the new cells being counted are being replaced i.e. the production factor This is seen to be particularly striking degree following adrenalectomy Perhaps one of the best examples is experimental radiation If a normal mouse is exposed to 200 R under specified conditions there is a striking lymphocytopenia and involution of lymphoid tissue 48 hours later the lymphocytes have not come back and the lymphoid tissue has only begun to return to normal size and structure But with the same dose of radiation in an adrenalectomized animal 24 hours later the lymphoid tissue has not only come back to normal size and structure but there is actually a lymphoid tissue hypertrophy Thus the observations we have been talking about are perhaps not surprising and a possible explanation it seems to me is that eosinophils in the blood represent only one of the variables

Thorn The interesting aspect of this experiment and one of the points I wanted to illustrate was that at the time the eosinophils were rising and one might say there was an escape from adrenal hormone effect there was a virtual absence of sodium in the urine Thus the 100 mg of compound F given around the clock did not permit the usual escape of sodium that might have been anticipated at this point

Long There are studies in the literature which show that very soon after the eosinophils fall in the blood there is marked stimulation of the eosinophilic elements in the bone marrow presumably the longer the eosinophils are lowered in the blood the greater the stimulation for the new production of eosinophils will be I believe Spears and the group at Wisconsin have published simultaneous bone marrow blood eosinophil counts To follow through on Dr White's suggestion it may well be that in the period of eosinophilia the bone marrow was intensely active in the production of eosinophilic cells

Sayers Another example where one gets into difficulties with indices when studying changes over a considerable period of time is changes in ascorbic acid concentration in the adrenal Initially following the application of a stressful stimulus a marked depletion of ascorbic acid occurs in the gland If the stimulus is applied continuously the ascorbic acid concentration will in many instances be restored to normal and even reach higher than normal concentrations Dr Long's group has shown that and we have had it appear in many instances in our own experiments I think what happens is that the gland synthesizes ascorbic acid at a more rapid rate

Long I think that is correct because in our experience and I believe in yours at such a time the cholesterol content of the gland will be much depressed and the gland will be enlarging although the ascorbic acid would lead one to believe that a normal gland is being dealt with

Pincus Do you follow the uric acid in these experiments?

Thorn We do follow the uric acid excretion but I don't have the values in this instance. We propose to bring this patient back and repeat the intravenous infusion of the same quantity of F without the operation in order to detect whether utilization or alteration in the metabolism of the hormone takes place

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